EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

PUBLISHED BY

THE SOCIETY FOR THE STUDY OF EVOLUTION

Vol. VIII SEPTEMBER, 1954 No. 3

PHYLOGENETIC ASPECTS OF RESPIRATORY FUNCTION

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Received November 27, 1953

Introduction

Respiratory mechanisms may be classified according to the following outline:

A. Environmental Medium (Krogh, 1941)
  a) Aquatic (sea of water—ocean, lake, etc.)
  b) Atmospheric (sea of nitrogen—troposphere, stratosphere)
B. Environmental Exchange (Krogh, 1941)
  a) Diffusion (molecular migration—microcosmic forces)
  b) Ventilation (breathing—macrocosmic forces)
C. Flow Pattern
  a) Circulatory (fluxional—continuous breathing exchange)
  b) Reciprocatory (tidal—reversal of breathing exchange)
D. Rhythm Frequency Pattern
  a) Periodic (intermittent ventilation cycles—closed system)
  b) Eupneic (intermittent complementary cycles—open system)

When the respiratory processes of animals are thus compared, it can be shown that a functional continuity persists throughout the kingdom; for the outline above describes the functional characteristics of respiratory exchange in order of the increasing complexity and evolutionary development of organisms. It can also be shown that elements of the more primitive respiratory types persist in the embryological development and in the adult life of the more specialized organisms. Thus there is a functional counterpart of organ development and modification which in comparative anatomy and embryology have been successfully described and utilized. It is, therefore, proper to discuss the phylogeny of respiratory function (Spurway *et al.*, 1953). Knowledge of phylogeny has predictive and utilitarian value in considering new problems of respiration.

Aquatic Respiration

The most primitive respiratory exchange is the aquatic diffusion type which involves no specialized organ. The generalized cell membrane, or an integument which is suitably permeable to respiratory gases, is adequate. Diffusion alone suffices for small aquatic or "humidic" organisms, such as aquatic protozoa, sponges, coelenterates and terrestrial or aquatic worms. However, even in protozoa, such protoplasmic projections as pseudopodia may be considered to sub-

serve a respiratory function by increasing the area of diffusion surface, and motility may be essentially a breathing activity. Comparative anatomists (Romer, 1950) have suggested that gill systems first served chordates as food gathering mechanisms and that their respiratory function was initially an accessory activity. This interesting possibility arises from the study of the Ammocoetes larva of *Petromyzon*, an organism which may represent the direct line of descent of chordates from invertebrates. Its gills are food collecting structures.

True respiratory organs appear concomitant with increase in body size and metabolic requirement very early in animal development. A typical aquatic respiratory structure is the gill filament, which is characterized by a) attenuated shape, b) semipermeable, delicate surface with large area in relation to its volume, and c) some type of circulation of body fluids in its core. The development of circulatory mechanisms and of transport media with respiratory pigments makes a separate, interesting phylogenetic story. Evidence for biomolecular evolution can be derived, for example, from comparative studies of the functional characteristics of hemoglobin (McCutcheon, 1952). Gill-like structures are found in many species of worms, arthropods, molluscs, echinoderms, and chordates. They are utilized for atmospheric respiration in a few animals, such as shore crabs (*Carcinus*) and certain tropical fish (*Hypopomus*) (Carter, 1931).

Aquatic and atmospheric (or aerial) respiration have been compared and the biological transition from one to the other has been critically reviewed by Carter (1931); the valuable work of Carter and Beadle (1930) on South American swamp animals considers fishes which seem phylogenetically transitional, with adaptations for the two basic respiratory media. Carter (1931) states, for example, "The comparison of lung and gill in *Erythrinus* is of interest. In this fish the gills are well developed and are able alone to maintain active life, when the water is well oxygenated. The vascular part of the air-bladder, the functional lung of this fish, can equally do so in completely deoxygenated water. Both are efficient respiratory organs for the whole body, but the vascular area of the air-bladder is more than one and one-half times as great as that of the gills." Such lungfishes have been considered ancestral to the Amphibia, in some categories (e.g. *Rana catesbiana*) of which the remarkable life history encompasses discrete functional specialization for each of the respiratory media; and in which during metamorphosis, these specializations, gill breathing and lung breathing, exist at the same time. A common ancestry for modern lungfishes and amphibians seems more acceptable to contemporary comparative morphologists (e.g. Romer, 1950). Such ancestors could be the *Devonian Crossopterygii*, judging from paleontological and fossil evidence and from the living specimens discovered in 1939 and in 1953. These latter specimens, taken alive, are coelacanths with compound respiratory function like modern lungfishes.

Atmospheric Respiration

The typical atmospheric respiratory structure is a pouch- or tube-like structure, such as the simple lung, the alveolus, or the tracheole. This has the same basic characteristics of surface as the gill filament, but it is an invaginated rather than evaginated structure; it contains nitrogen rather than body fluid, and it is surrounded by body fluid rather than water. However, it is essentially like an inverted gill; the surface exposed to gas—the respiratory surface—is wet like the gill's surface. Atmospheric respiration is, therefore, basically aquatic. Like aquatic respiration, the gaseous exchange betewen the environmental atmosphere (troposphere), or the pulmonary atmosphere (*pneumatosphere*), and body fluid or blood remains the elementary process of solution and diffusion, no matter how complex the intervening respiratory mechanism.

Diffusion

Atmospheric animals live in a *sea of nitrogen,* which contains dissolved O_2, H_2O, CO_2 and traces of other gases to form the solution called *air*. The compressability and the rapid water vapor diffusion characteristics of this gaseous environment impose complex requirements on respiratory mechanisms which are virtually non-existent in the physiology of aquatic animals. Aquatic animals live in a sea of water with such substances as O_2, CO_2, N_2, H^+, Na^+, K^+, Ca^{++}, Mg^{++}, OH^-, Cl^-, HCO_3^-, PO_4^{--} in solution and in various molecular combinations. This solution in the ocean resembles body fluids, and aquatic media generally include respiratory gases already in solution. Sea water was itself the probable inorganic basis of protoplasm, and it is a medium of striking similarity to all body fluids. The respiratory advantages to aquatic animals of ubiquitous water, a uniform, incompressible medium for the ventilation mechanism to pump, are offset a) by the relatively high density ratio of water to air (1000 : 1); b) by the relatively low solubility of gases in water (water saturated with air at 20° C. contains 0.55 vols. per cent O_2, compared with 20.95 vols. per cent in the air itself); and c) by the relatively slow diffusion rate of respiration molecules in water as compared to the diffusion of these molecules in nitrogen or air (e.g. the diffusion coefficient of O_2 at 20° C. in water is 0.000034 and in air is 11.0 (Krogh, 1941); *i.e.* O_2 diffuses in air in one second as far as it diffuses in water in about one week).

Atmospheric animals afford, in varying degrees, the cost of water conservation and of a more complex ventilation mechanism to handle a gaseous medium. They gain the advantages of rapid exchange and larger oxygen supply.

As long as elementary solution and diffusion suffice for an animal's demand for O_2, whether it lives in ocean or in air, elementary structures (evaginated filaments, invaginated tubes or sacs) and mechanisms suited to the elementary forces are sufficient. Gross behavioral activity, such as motility of a ciliate or locomotion of a frog tadpole to renew a depleted medium, is the extent of physiological regulation required for primitive aquatic diffusion respiration. Only valve action which can limit molecular diffusion at special respiratory openings, such as the pneumostome of snails, stomata of plants, and spiracles of insects, is the extent of regulation required for more specialized atmospheric diffusion respiration. In either environment, the quantity and pressure of CO_2 accumulated within the organisms may be utilized to regulate the exchange. In the atmospheric environment, however, water evaporation becomes a critical factor, so that a molecular compensating device (e.g. a regulated pore) is common among diffusion organisms with a high oxygen requirement.

Carter (1931) states from the work of Bohr that the speed of diffusion of CO_2 is much greater than that of O_2 in both air and water. That CO_2 diffusion is more rapid in air is doubtful, however, in view of Graham's law of diffusion (diffusion varies inversely with the square root of the density, or molecular weight). The Handbook of Chemistry and Physics (1948) gives the diffusion coefficients in cm^2./sec. for CO_2, at 0.0° C.—0.139, for O_2 at 0.0° C.—0.178, and for H_2O at 8.0° C.—0.239. Absolute values for the diffusion of H_2O in air from which direct comparison with O_2 and CO_2 can be made are not available to me. However, it is clear that the diffusion coefficients of these gases differ. If the gaseous diffusion of water vapor is uncomplicated by polymerization or other physical variability from strict molecular dimensions, then the pore offers a means only of altering vaporization losses to the optimum amount consistent with O_2 uptake or heat disposal requirements. Polymerization of water molecules in liquid state is well known, and this may occur at physiological temperatures in the vapor state. If so, then the regulated pore can be adjusted to utilize this difference and admit more O_2

molecules for each water molecule lost than present physical knowledge of ordinary gases considers. An experimental analysis of this possibility would be suggested by the finding of Schmidt-Nielsens (1950) that in desert rodents (*Dipodomys spectabilis; Dipodomys merriami,* kangaroo rats; and *Perognathus* spp., pocket mice) breathing dry air, the total evaporation was only about 0.5 mg. H_2O/ml. O_2 utilized, whereas for man the lung evaporation under similar conditions is about 0.84 mg. H_2O/ml. O_2.

The water conservation problem in atmospheric respiration may be emphasized by utilizing facts known about man in relation to a specific environment. The evaporation rate given by the U. S. Weather Bureau for an area of water in a pan four feet in diameter at a spot one-half mile from North Yakima, Washington, is 68 inches a year. The estimated area of the human lung varies from 50 to 100 m^2., say 100 m^2., or 155,000 in^2. (Nims, 1952) for easy calculation. Then (rounding all figures):

pan { Area of pan (πr^2)—1800 in^2.
Vol. evap./yr. (1800 × 68)—122,000 in^3./yr.

lung { Lung area/pan area (1550 × 100/1800)—86
Vol. evap./yr.—10,500,000 in^3./yr., or 170,000 liters/yr.

This is approximately 500 liters a day that would evaporate from the surface of the human lung if exposed for maximum O_2 diffusion, like the gills of Necturus or early frog tadpoles, at this location. The actual water loss from the lung of man (500 ml./day) is about 1/1000 of this amount.

Not only are specialized respiratory surfaces internal, but specific mechanisms for regulation of exchange at respiratory openings are present in atmospheric breathers. Such obvious mechanisms are the spiracular valves of insects, the pneumostome muscles of gastropods, and the valves of the nares and glottis of amphibians and reptiles. Less obvious, but potentially analogous, are the intrapulmonary sphincters of birds and mammals.

Ventilation

Diffusion exchange even limits the size of active cells, and it is too slow for the demands of metazoan animals which require large and rapid flow of oxygen (Harvey, 1928). Supplementary ventilation of the respiratory membranes by special mechanisms more than offsets, by additional oxygen supply, the increased energy output required to move the medium, and a variety of such mechanisms characterize both aquatic and atmospheric breathing organisms. This leads to consideration of flow and rhythm patterns.

Flow Pattern

Breathing in aquatic animals may occasionally be a) *reciprocatory* (or tidal) with an in and out movement of water, as in a few animals like coelenterates, echinoderms, or cephalopods, but usually it is b) *circulatory* (or fluxional) with a continuous direction of water movement, as in most aquatic breathers like lobsters or fish. In atmospheric breathers, by contrast, reciprocatory breathing is the most prevalent among various classes, although circulatory breathing is characteristic of the two types of terrestrial animals which have the highest oxygen requirement (metabolic rate) in their respective classes, namely flying insects (Lee, 1925) and birds (Hazelhoff, 1943). In birds, though the environmental exchange is reciprocatory, the flow past air capillaries of the lung, which are analogous to mammalian alveoli, is circulatory. The circulatory type, therefore, may be considered the most specialized breathing type in air.

The inefficiency of tidal breathing for aquatic animals is obvious from the magnitude of forces required, when comparing water and air, to reverse the direction of flowing water. The density of water is more than one thousand times that of air. A unidirectional flow of water is much less costly to maintain, and certain fishes (e.g. mackerel) may even utilize their locomo-

tion to maintain this flow over their gills (Hall, 1930). Tidal breathing, however, has relatively low energy cost in the atmosphere (5.5 cc. O_2 to move a liter of air. Schneider *et al.*, 1948).

Periodic Rhythm Frequency Pattern

Breathing movements, inspiration and expiration, may be either *periodic* or *eupneic* (see fig. 1). Periodic, or intermittent, breathing characterizes amphibians (Willem, 1920), reptiles (Boelaert, 1941, 1942) and parafoetal birds (Romijn, 1948) and foetal mammals (Barcroft *et al.*, 1936; Windle, 1941; Cross *et al.*, 1952). This pattern has been recognized in resting alligators (Boelaert, 1942) and turtles (McCutcheon, 1943), in which a

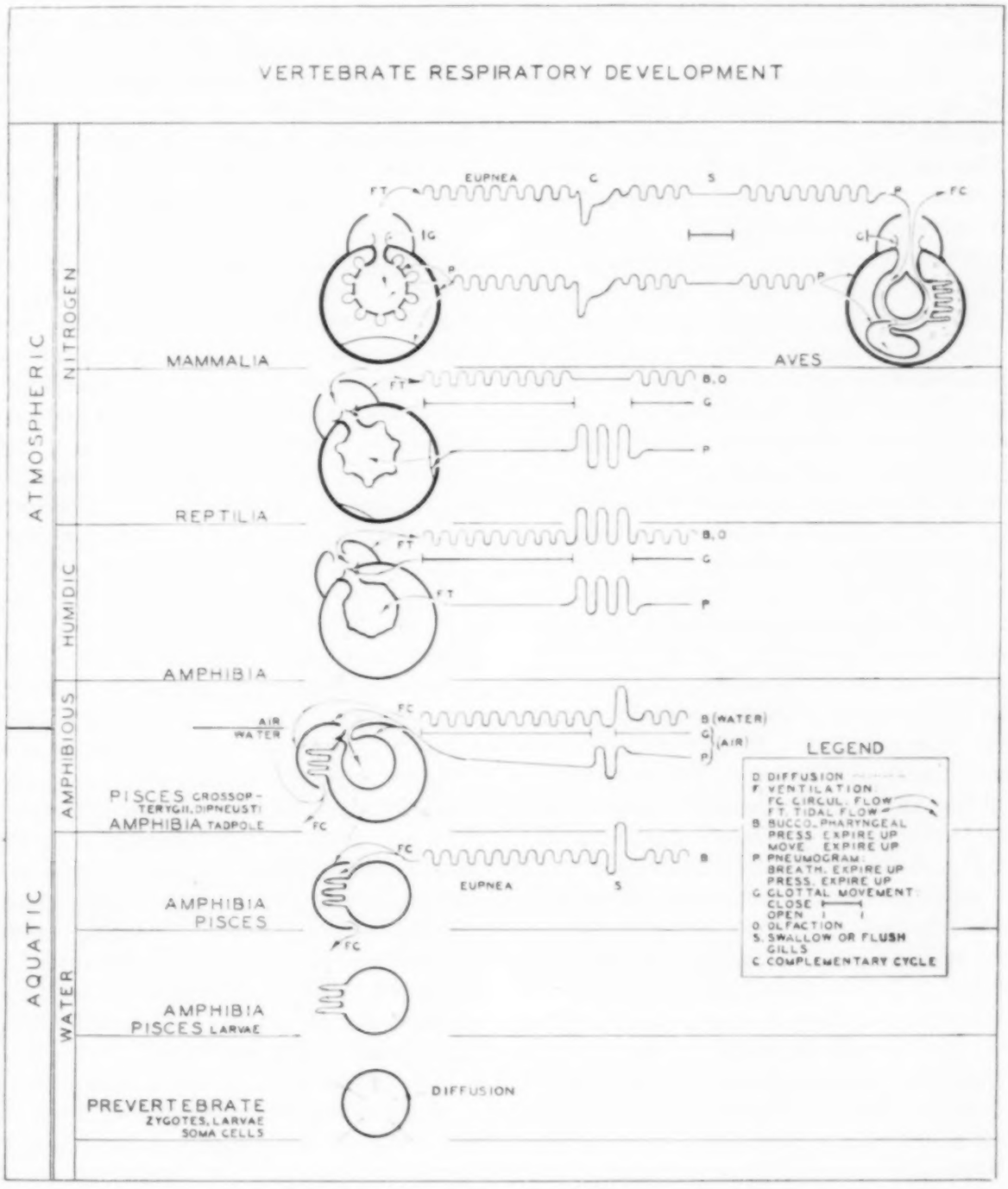

FIG. 1. Schematic diagram of features in respiratory physiology related to vertebrate categories and environmental media.

series of breathing cycles, typically two or three, is followed by a period of apnea which may last much longer than the breathing period. A eupneic pattern of breathing is, however, superficially apparent in amphibians, like the frog. In frogs the most obvious feature of breathing is the throat movements which force air into the lungs, and these movements have a continuous eupneic pattern. However, these throat movements also subserve olfaction. Closure of the glottis after a period of breathing cycles effectively closes off the lung and imposes a periodic pattern on breathing *per se* (Willem, 1920). The continuing throat activity only affects the movements of air across olfactory membranes, not breathing.

The lungs of the frog, and even of reptiles, are relatively simple sacs compared to the complex alveolar lungs of birds and mammals. One of these lungs in a frog has been likened to a simple unit of the mammalian lung—that is, to a bronchiole and alveolus. Structures for intrapulmonary flow regulation are lacking. External regulation through glottis closure in amphibians and reptiles, however, has large significance for respiratory exchange in three respects, a) regulation of diffusion, b) regulation of filtration, and c) regulation of pressure. It effectively regulates the period of exchange of H_2O, O_2 and CO_2 between pneumatosphere and atmosphere, and this in turn permits some mechanical stability for the exchanges by diffusion at the respiratory membranes. By such isolating action, stabilized CO_2 concentration can eventually become a primary regulator of breathing, as in birds and mammals. Both O_2 and CO_2 concentrations seem to be equally important regulators in amphibians, in which the skin respiratory exchange is large and not subject to this type of regulation. O_2 regulation dominates in aquatic animals (Bishop, 1950). Water loss is minimized by glottal closure in reptiles, which have negligible skin diffusion. Diffusion out of the lung is blocked and there is no necessity to saturate large volumes of flowing air without optimum extraction of its O_2 content.

Closure of respiratory openings provides physiological regulation over intrapulmonary (pneumobaric) pressure. The lung is the only place in an animal's body where capillaries come into such intimate relationship with atmospheric pressure. At all other tissues there is a small, positive tissue fluid pressure maintained by the elastic properties of enclosing connective tissues and ultimately by the integument. This tissue fluid pressure, though small, is a counterbalance, along with protein osmotic pressure, against filtration hydrostatic pressure in the vessels (Evans, 1952). Increased filtration from capillaries into tissue fluids tends to increase the tissue fluid pressure itself, and the loss from vessels becomes self-limiting at usually minor cost to overall water balance. In lungs the circumstances attending fluctuations in filtration pressure are much more critical. If capillary pressure rises, the increased filtration into pulmonary respiratory spaces is unopposed by elastic membranes. It is not self-regulating through tissue fluid pressure. But a large gas-fluid inter-phase must be maintained or respiration will fail. Encroachment of filtration fluids on this space can be only slightly tolerated. In a closed pulmonary (or tracheal) system the pressure of the pneumatosphere (pneumobaric pressure) can be maintained through action of respiratory muscles at a level sufficiently high to counterbalance filtration tendencies. Average pressures well above atmospheric have been clearly shown in lungs of turtles (McCutcheon, 1943) and in tracheae of flying insects (McCutcheon, 1940; Watts, 1951). There is suggestive evidence for such pressures in the alveoli of terrestrial mammals (McCutcheon, 1951), but direct measurement has not been possible.

A third importance for a positive pneumobaric pressure, which is facilitated by closure of the glottis, is an increased oxygen pressure gradient toward the blood. This could be of value in times of respira-

tory stress from environmental O_2 deficiency or excessive metabolic demands, and during dives or other periods of apnea. It would have small significance in normal exchange.

Unlike amphibian breathing, which is of a force-pump character, the breathing of reptiles, birds, and mammals is of an aspiration-pump type. Air is drawn into or enters respiratory spaces by enlargement of the body cavity. In reptiles, closure of the glottis persists, as in amphibians, to isolate the pneumatosphere during periods of apnea. Throat movements also persist in reptiles during apnea, but these also must function primarily for olfaction (McCutcheon, 1943; Root, 1949).

Olfaction is a primitive sense of primary importance in lower vertebrates, as evidenced by the very large size of the olfactory lobes when compared with other parts of the brain. Olfaction depends on contact of air-borne molecules with specialized olfactory cells. This limits the efficacy of the sense where only diffusion, or diffusion respiration, prevails. Active fanning of the membranes to greatly increase molecular sampling is as obvious an advantage for olfaction as for breathing, and periodic breathers utilize the throat breathing mechanism for this accessory function. The rhythmical character of reptilian throat movements led many biologists (e.g., Agassiz, 1857; Potter, 1938) to erroneously consider them, by analogy with the frog, to be breathing movements. Glottis closure makes this untenable, and their small respiratory significance can only be associated with reduction of dead space and a minor exchange of O_2, CO_2, and H_2O across nasal and buccal membranes. These membranes are not usually adapted for respiration.

Various muscular membranes of the body wall and intracoelomic septa are utilized to create breathing forces in animals. Turtles, because of the shell, have a somewhat unique mechanism. The body cavity is enclosed in a rigid box which has the ribs rigidly incorporated in it, and there is no homolog of the mammalian diaphragm. The membranes at the shell openings provide the necessary force and distensibility (McCutcheon, 1943). Glottal closure during apnea is an advantage for aquatic habitat, for it releases inspiratory tonus and frees musculature for locomotion during dives. The pulmonary diaphragm of birds contracts on expiration, rather than on inspiration like the mammalian diaphragm (Hazelhoff, 1943).

Eupneic Rhythm Frequency Pattern

In contrast to the *periodic* cycles of primitive air breathers, the specialized, homoiothermic birds and mammals are characterized by an *eupneic* breathing pattern. Also in contrast to the normally closed glottis of amphibians and reptiles, the glottis of homoiothermic animals is normally open. Regulation of exchange, diffusion, and pressure, which is accomplished by the valves of nares and glottis in poikilotherms, must reside, in homoiotherms, in structures distal to the glottis. The myoelastic tissues of bronchioles and of mouths of air sacs and alveoli are structurally such potential regulators (Macklin, 1929, 1950; Miller, 1947). Their autonomic innervation is also consistent with this function. There is some indirect evidence that they do, in fact, help regulate diffusion, volume (Verzar, 1946; Peyser *et al.*, 1950), and pressure (McCutcheon, 1951), but their direct study has been so limited by technical and biological difficulties that unequivocal statements cannot presently be made. Some physiologists consider their function to be mainly mechanical, particularly guarding against dust particles and the like, but this seems much too limited a view.

Recent studies (McCutcheon, 1951, 1953) of mammalian breathing have shown that a continuous eupneic breathing pattern does not exclusively prevail. There is a persistence of elements which are characteristic of the breathing patterns of poikilotherms in the form of periodically recurring, deep, sigh-like or gasp-

like cycles, which are called *complementary cycles.* Such cycles have long been observed in mammals when subject to high CO_2 or low O_2 concentrations (Haldane *et al.,* 1919a), to restriction of abdominal movements (Haldane *et al.,* 1919b), to various drugs and anesthetics (Breckenridge *et al.,* 1952), circulatory failure, respiratory center injury (Hoff *et al.,* 1952) and similar respiratory stresses. Emphasis on the occurrence of complementary cycles as a normal breathing entitity, however, favors the recognition in the respiratory process of a biogenetic and evolutionary sequence of function.

There persists at the highest level of respiratory specialization, in the rhythmical eupneic breathing of birds and mamals, elements of the more primitive respiratory types a) diffusion, and b) periodic ventilation. A reversion to a more primitive respiratory pattern occurs in these animals under stress, as higher levels fail. The developmental succession of these types: namely, from primitive diffusion in ovum and embryo, or foetus, through abortive ventilation movements in parafoetal (bird) or foetal (mammal) stages, to periodic breathing in premature or new-born before rhythmical eupneic ventilation is established—this is the pattern of development and of persistence of primitive elements in related species which also characterizes important generalizations about structural elements, like pro-, meso-, and metanephric kidneys, in comparative anatomy and embryology. Here may be recognized a) vestigial or rudimentary functions (McCutcheon, 1953), b) progressive levels of functional specialization among species, c) regression under stress to more primitive levels of function, d) developmental sequence through more primitive processes to achieve specialized functions (Barcroft, 1938).

Breathing in atmospheric animals subserves numerous ancillary functions. Olfaction has already been mentioned. It is suggested that the neurological basis (premedullary) for rhythmical eupneic, panting, and sniffing movements in mammals may be the mechanism regulating breathing throat movements in fishes and olfactory accessory throat movements in amphibians and reptiles. The periodic complementary cycles of mammals are probably regulated by the more primitive gill clearing and periodic breathing centers (medullary) which are dominant in poikilothermic vertebrates.

With the advent of homoiothermism (Rodbard, 1953) in animal development, breathing acquired a thermoregulatory value. Pulmonary evaporation (i.e. panting) is a primary source of thermoregulation in birds and in most mammals; it is secondary to the integument in some mammals like the horse and man. In thermoregulation, a large, ventilated, evaporative surface is important. The breathing structures are exceptionally well suited, but the cost of such cooling in terms of permissible water loss and CO_2 fluctuations needs to be accounted. Calories lost in terms of O_2 molecules required would be secondary, since conditions favoring H_2O evaporation also favor O_2 uptake. But adjusting the unavoidable loss of H_2O during O_2 uptake to the optimum O_2 requirement appears to be a biological demand on homoiothermic animals generally, and a specialization for desert life (Schmidt-Nielsen, 1950). Panting animals afford the cost on respiratory function alone, whereas the few sweating animals divide it between respiratory function and the integumentary functions of insulation and mechanical protection.

Other ancillary functions of the breathing mechanism are a) phonation and b) compression of visceral contents. Phonation, which depends on pressure and flow variations of lung contents, is highly developed only in birds and mammals. For the phonation of amphibians and reptiles, accessory air structures occasionally develop, such as special vocal sacs. Lacking these, the lung air may only occasionally

be utilized, as in the hiss of alligator, snake, and turtle.

It appears that complex phonation, and also panting, could develop in birds and mammals only when large volumes of respiratory gas became structurally and functionally isolated from the exchange areas of the lung. How this is accomplished in birds can be recognized from the presence of large air sacs and unidirectional mass flow of gas in the various bronchi. Diffusion exchange from parabronchi in and out of air capillaries (cylindrical alveoli; Cover, 1953) is separated from the ventilation of main reservoirs and the mass flow of gas (Hazelhoff, 1943). That an equivalent separation of the flow and exchange areas can be accomplished in mammals is less obvious, for the alveoli usually lie at the ends of the airways rather than appendant along their course. Very few mammalian alveoli arise directly from bronchiolar walls, as in birds. Most of them arise from the air sacs, which are too often confused in physiological discussions with true alveoli. These sacs constitute reservoirs along the direct course of mass air movement in the passages—movement up to but not through the sphincter pores of alveolar openings. It is these sacs, however, which would appear to provide, along with elongation of the pulmonary airways, for gross volume changes through mass flow and to provide a reservoir for regulated diffusion exchange with the alveoli (Haldane, 1919). The alveoli themselves probably do not cyclically alter in volume during eupnea or panting.

An ecological analogy may help to clarify the apparent ventilation differences between bird and mammal. Let a mountain stream represent the air flow in a bird's bronchi, and let a small pool connected to the stream by a rock fissure represent its alveolus, or air capillary. Plankton in the stream can represent O_2 molecules. The plankton organisms are swept along the course of the main stream by the water flow, but they may migrate through the fissure into the pool by locomotion. The air flow in a mammal's system differs, as represented by the flow in a bay connected by a gut with the ocean. An alveolus may here be represented by a seepage pool bordering the bay. Plankton organisms then would be carried in and out of the bay by the tidal ebb and flow, but again, like in the mountain pool, they could migrate through the seepage channels into the pool by locomotion. In either case the main flow, streaming or tidal, carries the mass of organisms, or molecules,, passively along the major course; but the exchange with the pools, or alveoli, is only indirectly affected by this major flow. The basic exchange is by active migration (diffusion) in either case.

It seems clear that a tranquil *pool of nitrogen* has been achieved in birds and mammals (and insects) for their internal respiratory medium. Roos *et al.*, 1954, has recently emphasized a "relatively stagnant 'diffusing' area" in man. The mechanical, physical, and chemical hazards which plague an externally exposed respiratory organ, like the gills of amphibians, are reduced to a minimum by sequestered location and physiological control of pneumatosphere in pulmonary units. The pneumatosphere is subject to regulation of pressure and volume; it constitutes a physical buffer between a) the respiratory demands for uniform diffusion of gaseous molecules within the alveoli and b) the olfactory, thermoregulatory, compressatory, and phonatory demands for grossly fluctuating and harshly altered movement of masses of gas between animal and atmosphere. A second order of regulation of breathing determines the exchange between the pulmonate animal's pneumatosphere and the atmosphere. This regulation is represented through strategic structures, such as nares, glottis, bronchioles, hairs, mucous glands, and cilia; and through controlled actions, such as apnea, hyperpnea, panting, sniffing, sighing, gasping, yawning, coughing, and sneezing (Comroe, 1953).

It is in the evolution of progressively

more complex and refined respiratory mechanisms that the necessary conditions for such highly specialized activities as bird's locomotion and man's cerebration have been developed. Essential features of respiratory development are indicated in Figure 1. Through this evolution an adequately supplied and critically regulated oxygen content in the internal environment is achieved. This is the most temporally urgent requirement among all of Claude Bernard's conditions for a free and independent existence—for flight of body or flight of fancy.

Summary

Functional characteristics of respiratory exchange, which parallel structural evolution, can be traced from primitive *aquatic diffusion* respiration through *atmospheric diffusion* respiration to *atmospheric ventilation* respiration. Ventilation respiration can be similarly traced from aquatic, *circulatory* flow mechanisms to atmospheric, *reciprocatory* flow mcehanisms. Flying insects and birds are exceptional among atmospheric breathers in their deveolpment of a modified circulatory ventilation which may be correlated with metabolic requirements of aerial locomotion.

The dominant reciprocatory flow pattern of atmospheric ventilation evolved from the *periodic* frequency patterns of amphibians and reptiles to the *eupneic* frequency pattern of mammals. Amphibians and reptiles have a *closed pulmonary system* with a predominantly diphasic or triphasic breathing rhythm and long intermittent periods of apnea. Mammals have an *open pulmonary system* with a dominantly eupneic breathing rhythm and intermittent complementary or sigh-like cycles.

Glottal regulation in amphibians and reptiles (and spiracular regulation in insects) provides a primitive, physiologically determined pulmonary atmosphere (pneumatosphere). This reaches the most specialized homeostatic condition in birds and mammals (and in flying insects) through regulation of intrapulmonary sphincters (or tracheolar distribution) and through functional isolation of the diffusion medium (nitrogen) from the mass air flow involved in ventilatory, olfactory, thermoregulatory, phonatory, or compressatory activity.

Ontogenetic repetition of many elements in the evolution of breathing is evident in mammals. Also the specialized respiratory function of higher animals contains rudimentary or vestigial elements of the primitive exchange and regulatory processes. Respiratory exchange may degenerate to these more primitive processes under stress.

Comparative and developmental physiology of respiration indicates an evolutionary pattern of function which parallels morphological and systematic patterns of evolution. The respiratory evolution provides the most immediately urgent homeostatic requirement for the most highly specialized of animal activities— aerial locomotion and cerebration.

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FURTHER SEROLOGICAL DATA ON THE RELATIONSHIPS OF SOME DECAPOD CRUSTACEA

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Received March 20, 1954

INTRODUCTION

To identify one kind of protein molecule as being more primitive than another kind is more difficult than making the same determination for a skeletal structure, because the paleontological record tells us little or nothing about protein molecules. Both the paleontologist and comparative morphologist are concerned with establishing homologies [2] among organisms. They recognize structural similarites on the basis of comparisons among visible morphological structures, and almost all of the taxonomic information on organisms has been accumulated from morphological and paleontological studies. In recent years, however, studies in experimental biology have yielded evidence suggesting that there are molecular and submolecular homologues. The influence that this newer type of information will have upon our understanding of taxonomy and evolution is still to be realized.

The results of physiological and biochemical studies indicate that many seemingly identical enzymatic, protein molecules have a widespread distribution among the organisms of the world. In such instances the concept of molecular homology has implications for a better understanding of the evolution from a nonliving to a living system, and for our appreciation of the probabilities of a monophyletic origin or of polyphyletic origins of life. These indispensable molecules show us, in a very precise way, the solutions of problems of survival which all living substance have had to reach in order to persist and evolve. The application of this type of information on molecular homology to problems of evolution in the more limited sense, that is, of a particular Class or Order of organisms, seems at present, to have little value.

The study of genetics, as is well known, has provided us with an understanding of some of the physical mechanisms, causes, and results of evolution. In addition, the synapsis of chromosomes has given us visbile evidence that genic homologues exist within the nuclei of cells. Dobzhansky (1951) and Spencer (1949) have advanced evidence for interspecific gene homologies in the genus *Drosophila*. Whether genes are considered merely as function concepts, or as nucleoplasmic realities, the precise location within the chromosome of each self-perpetuating determinant is indisputable. Here then, is

[1] This study was aided by a contract between the Office of Naval Research, Department of the Navy and the University of Kansas, NR 162-013.

[2] As pointed out by Boyden (1947) an unfortunate confusion exists in the use of the word homology. Since comparative morpholgists, paleontologists, geneticists and serologists all use the word or its derived adjective, the various definitions as prepared by Boyden are quoted or paraphrased here in the hope that they will permit mutual understanding among the workers in the various disciplines:

a. An essential similarity in the structure and development and in the relative position and connections of corresponding parts of the bodies of organisms.—General.

b. An essential structural similarity due to common ancestry.—Paleontologists.

c. The relationship between chromosomes which pair during maturation or which are descended from the same original chromosomes. —Geneticists.

d. The relation among genes acting as alleles, or between corresponding loci on similar chromosomes.—Geneticists.

e. The relation between an antigen and the antiserum produced in response to it.—Serologists.

another instance of molecular, or submolecular, homology of undoubted significance in evolution. Cytological evidence for the existence of homologous bands of chromatin in related species is a direct indication that corresponding morphological structures do result from the action of the same or similar genes on similar kinds of cytoplasm.

Living organisms can be interpreted as a successful accumulation of their genetic potential. When there are good paleontological records, the relative similarities and differences among the special homologues of related organisms can be compared directly. Even when the paleontological evidence is lacking, we can logically reason that the same genetic mechanisms were involved in the past as are functioning today, and that special homologues of recent organisms reflect in some degree their genetic similarity. The serum proteins of organisms are products of evolution just as are the proteins which compose the morphological characters of the body. Results of serological comparisons of serum proteins usually verify the better classifications of organisms which are based on both morphological and paleontological evidence.

In serological comparisons among the corresponding proteins of related species of organisms the reaction between a protein and the antiserum produced against it (the homologous reaction)[2] at least equals, and usually exceeds the reaction between this same antiserum and any other protein (the heterologous reaction). A heterologous serological reaction is interpreted as being indicative of some degree of similarity among the native proteins being compared. The amount of reaction is in proportion to the extent that the heterologous proteins resemble those against which a given antiserum was made. In this sense, serological comparisons measure chemical and structural homology at the molecular level. One might expect occasionally to come across native, heterologous proteins, whose antigenic radicals would combine with the antibodies of an antiserum so as to exceed the reaction of the homologous proteins. This does not happen. The constancy with which the phenomenon of quantitative serological specificity occurs argues in favor of a conservative and selective evolution of proteins in animals.

Recent serological experiments (Boyden and Gemeroy, 1950) have shown that arrangements of mammals based on comparisons of their serum albumins in one instance and their serum globulins in another were closely similar. The antisera made against serum albumins generally placed the mammals much closer together than did the antisera against serum globulins. These findings were interpreted by the authors as indicating a more conservative inheritance, hence evolution, of albumins than of globulins.

Among the Crustacea, where there is but a single kind of antigenic serum protein, the hemocyanins (Allison and Cole, 1940; Clark and Burnet, 1942; Tyler and Sheer, 1945), the problem of determining serological relationships is at the same level of refinement as the above-mentioned inter-albumin, and inter-globulin comparisons. The sera of Crustacea provide naturally occurring, relatively simple systems of corresponding proteins which may be compared directly, without chemical preparation in the laboratory.

In this paper I shall present serological arrangements of widely separated groups of decapod Crustacea and compare these results with classifications based on morphological and paleontological evidence.

Materials and Methods

Collection of antigens: The sera of the decapod Crustacea used in these experiments were collected over a period of years. Table I summarizes the information on these antigens. It can be noted from the table that the collections date back to the year 1932. It has been shown (Leone, 1949) that hemocyanins can be successfully stored in liquid form for periods of up to fifteen years without alter-

TABLE I. *Species compared and their geographic locality*

Species	Locality	Year Collected	Collector
Libinia emarginata Leach	Woods Hole, Mass.	1950	Leone
Maia squinado Rondelet (Herbst)	Plymouth, England	1939	Boyden
Cancer pagurus Linne	Dinard, France	1948	Leone
Cancer borealis Stimpson	Woods Hole, Mass.	1932	Boyden*
Callinectes sapidus Rathbun	Delaware Bay, N. J.	1947	Leone
Eriphia spinifrons (Herbst)	Naples, Italy	1948	Leone
Pachygrapsus marmoratus Fabricius	Banyuls-sur-Mer, France	1948	Leone
Calappa granulata Linne	Banyuls-sur-Mer, France	1950	Leone
Dromia vulgaris Milne-Edwards	Naples, Italy	1950	Leone
Pagurus pollicaris Say	Woods Hole, Mass.	1947	Leone
Dardanus arrosor (Herbst)	Naples, Italy	1948	Leone
Pagurus prideauxi Leach	Banyuls-sur-Mer, France	1948	Leone
Pagurus bernhardus (Linne)	Dinard, France	1948	Leone
Homarus americanus Milne-Edwards	Woods Hole, Mass.	1932	Boyden
	Long Island, N. Y.	1946	Leone
	Woods Hole, Mass.	1949	Baier**
		1950	Leone
Homarus vulgaris Milne-Edwards	Plymouth, England	1939	Boyden
Panulirus argus (Latreille)	Tortugas, Florida	1936	Boyden
		1939	Boyden
Palinurus vulgaris Latreille	Dinard, France	1949	Leone

* Dr. Alan A. Boyden, Director, Serological Museum, Rutgers University, New Brunswick, New Jersey.

** Dr. Harold Baier, Camp Dietrick, Maryland.

ation from the original native state insomuch as their antigenic activity is concerned. Identification of the species which were collected were made from the classifications and descriptions of Bouvier, 1940; Pesta, 1918; and Rathbun 1925, 1930, 1937.

Preparation of antigens: The bloods of the Crustacea were removed from the bodies of the organisms by two principal methods. For large organisms, one of the walking legs, usually the fifth periopod, was removed as close to the cephalothorax as possible and the animal was suspended over a receptable such as a porcelain pan into which the blood drained and then clotted. For small organisms the exoskeleton was punctured at the bases of the appendages (the periopods) with a 20 gauge needle and the blood withdrawn from the hemocoel into a syringe, and then emptied into containers and permitted to clot. Serum did not always readily separate from the clots. When centrifugation at 3000 rpm for 30 minutes would not yield the serum of a given sample, the clot was wrapped in several thicknesses of fine cheesecloth and squeezed by hand. The serum usually came out easily leaving behind a sticky, elastic, fibrin-like residue. Sera were either immediately sterilized through a Seitz filter and stored in serum vials in the refrigerator, or first preserved with chemicals and, at a later date, sterile-filtered and stored.

The chemicals which were used to preserve the sera of the Crustacea were Phenyl-mercuric nitrate in a final dilution of 1 : 10,000 (0.01 per cent), 'Merthiolate,' Lilly (Sodium-ethyl-mercurithiosalicylate) in a final dilution of 1 : 10,000 (0.01 per cent), or formalin in a final dilution of 1 : 500 (0.2 per cent—calculated on the basis of commercial formaldehyde being equivalent to 100 per cent formalin). Reciprocal serological tests were conducted, using preserved and native portions of serum from the same animal and serum from other animals of the same species, in order to test the influence of the chemicals on the serological characteristic of the antigen or on the specificity of the anti-

sera. For the mercurial compounds no significant serological effects have been noted to date. In the use of formalin as a preservative for hemocyanin, it has been found that a final concentration of 0.2 per cent will cause a slight, but hardly significant, increase in the precipitate-forming characteristic of the antigen (Leone, 1953). In the experiments described in this paper only the sera of the Paguridea (hermit crabs) have been formolized. It is unlikely that the formalin has invalidated the comparisons of the pagurid proteins with the other antigens.

Preparation of antisera: All of the antisera were prepared in rabbits. Several injection-schedules were employed in the production of antisera to the hemocyanins. The usual route of injection was in the marginal vein of the ear. When only a small quantity of antigen was available subcutaneous injections in the nape of the neck were employed. A series of injections consisted of four increasing

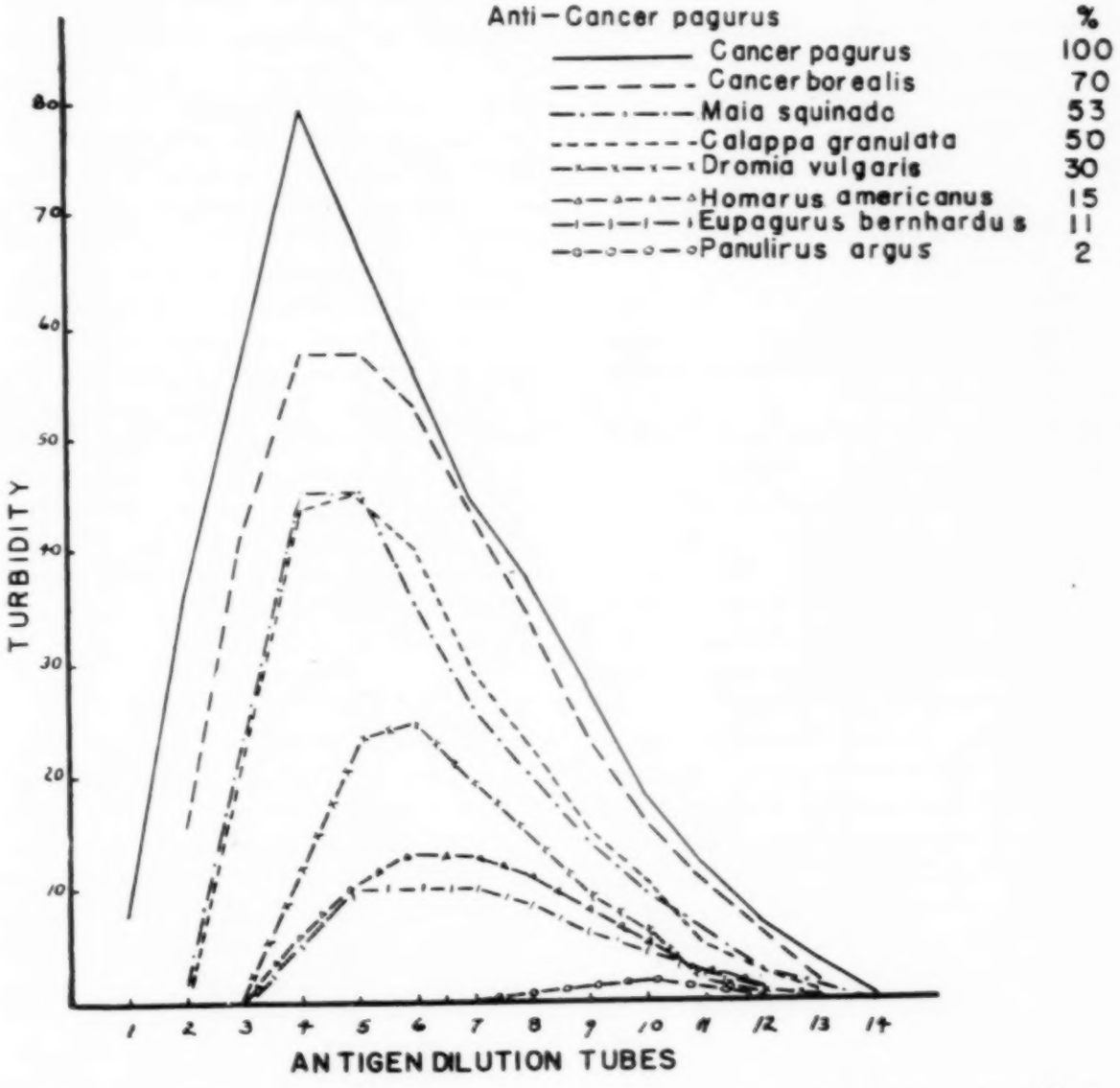

FIG. 1. A typical series of precipitin curves obtained with the sera of several species of decapod Crustacea tested with an antiserum produced against one of them. The summated turbidity values of the homologous curve is designated as the 100 per cent reaction. The cross-reactivities of the proteins from heterologous species are compared to the homologous reaction. Percentage values within 5 per cent of one another are considered to be of the same order of magnitude.

Eupaguras bernhardus = *Pagurus bernhardus* in text.

doses given on alternate days. The rabbits were trial-bled from the main artery of the ear 7 to 8 days after the last injection. If the antisera proved to be too weak, additional series of injections were given. Final bleedings were accomplished by cardiac puncture with an 18 gauge needle and a 50 ml. syringe. Twenty-four hours before each bleeding, the food of the rabbit was removed in order to clear lipids and other substances from the blood.

After being permitted to clot, the bloods of the rabbits were centrifuged and the antisera obtained. The antisera were sterilized by filtration through a Seitz filter, bottled in serum vials, and stored in the refrigerator. Some antisera were used after being stored for as long at 3 and 4 years, and, despite greatly reduced antibody activity, still had strong discriminatory reactions with a wide variety of antigens.

Method of testing: The serological method employed to measure the amount of reaction between antigens and antisera has been described in detail elsewhere (Boyden, 1926; Boyden and DeFalco, 1943; Bolton, 1947; Leone, 1949). Briefly the technique involves the use of a photoelectric turbidimeter designed by Libby (1938) to measure white precipitates of turbid systems. Each antigen is standardized in terms of amount of protein. Doubling dilution-series of known protein concentrations are prepared for each antigen. To each dilution a constant amount of antiserum is added. The antigen dilutions are usually prepared to extend from extreme antigen excess to extreme antibody excess. The combination of soluble antigens and their precipitins is an optimal-proportions reaction and the precipitates which form, start at a minimum in the region of antigen excess, rise to a maximum in the region of optimal-proportions, and decline to a minimum again in the region of antiserum excess. A polygon plot of the precipitates which form reveal a normal distribution skewed in the region of antiserum excess (fig. 1).

All dilutions of antigens were made with 0.9 per cent NaCl buffered with M/150 KH_2PO_4 and M/150 Na_2HPO_4 to pH 7.0 ± 0.1. 'Merthiolate,' in a final dilution of 0.01 per cent, was added to the buffered saline to serve as a bacteriostatic agent during the incubation of the antigen-antibody reactions. Twenty-four hours incubation at 20–22° C. was regularly employed as the reaction-time in these tests.

With each antiserum, the homologous antigen and a series of heterologous antigens were tested. A statistical value (the summated turbidities) which is proportional to the area of the precipitin curve was used as the index of the reaction. The "area" of the curve for the homologous reaction established the 100 per cent reaction-value for each antiserum. The order of reactivity for a series of heterologous antigens tested with a given antiserum was determined by calculating the "area" of the curve for each heterologous reaction and relating it in terms of per cent to the "area" value for the homologous reaction. Figure 1 shows a plot of a typical series of precipitin tests. Inasmuch as the amount of protein for the corresponding points on the curves was the same for all antigens, and the amount of antiserum was constant for all dilutions, differences in the amount of precipitate at any dilution reflect qualitative differences among the antigens.

Experimental Results

The data in table II summarize the results of the serological comparisons of the sera of seventeen species which served to represent six Tribes of decapod Crustacea. The table is arranged to conform to Calman's (1909) classification of the Crustacea. Each antiserum, with its own specificity, ranked (vertical columns, table II) the heterologous antigens in accordance with their degree of correspondence to the homologous antigen. When several antisera were produced against the proteins of one species, the relative orders of relationship of the heterologous antigens had a good positive correlation. The six antisera produced against the

TABLE II. *Serological relationships among some decapod crustacea.*

TABLE II. *Serological relationships among some decapod crustacea* [5]

Classification of crustacea (Calman, 1909)			Antigens	Antisera: *Libinia emarginata*	*Cancer pagurus*				*Cancer borealis*	*Callinectes sapidus*	*Pachygrapsus marmoratus*	*Calappa granulata*			*Dromia vulgaris*				*Pagurus pollicaris*	*Pagurus bernhardus*	*Pagurus prideauxi*			*Homarus americanus*						*Homarus vulgaris*	*Panulirus argus*					*Palinurus vulgaris*
Section: Brachyura	Tribe: Brachygnatha	Sub-tribe: Oxyrhyncha	*Maia squinado*	14	53				60	30		18			4				16	1	9			21	39	43				23	4					1
			Libinia emarginata	100		70			55		43	20	27		14	12			10		9						18				3	22	32			
		Sub-tribe: Brachyrhyncha	*Cancer pagurus*		100	100	100	100	75				24	32				14	19		8	9	5					11	11			19	22	9	7	
			Cancer borealis	88	70				100	65	31	18			5	12			19		8	9	5					11	11			19	22	9	7	
			Callinectes sapidus						65	100									32	5	12			15	37	31	17			15						15
			Eriphia spinifrons							61									22	4	7			20						20						3
			Pachygrapsus marmoratus								100								17	1	5			14												3
	Tribe: Oxystomata		*Calappa granulata*	14	50	80	63	61	47	30	51	100	100	100	13		6	21	19	1	9	9	7	31	45	50	22	20	14	30	10	19	33		13	1
	Tribe: Dromiacea		*Dromia vulgaris*	8		51	35	33	32	20	19	0	6	19	100	100	100	100	7	1	1	2	6	11	22	27	14	13	9	13	21	13	37	14	23	1
Section: Anomura	Tribe: Paguridea		*Pagurus pollicaris*							15									100	37	43			10	14	23				18	0					2
			Dardanus arrosor																27	34	48			9						9						
			Pagurus prideauxi	4	11	41	34	32	21	12	12	0	5	9	3	8	7	5	29	34	100	100	100				18	15	13			11	13	9	7	
			Pagurus bernhardus							10										100	56			7						7						
Section: Astacura	Tribe: Nephropsidea		*Homarus americanus*	4	15	33	18	19	23	21	19	2	8	20	13	4	7	15	7	1	7	9	9	100	100	100	100	100	100	85	2	23	39	20	23	18
			Homarus vulgaris							21														88						100						
Section: Palinura	Tribe: Scyllaridea		*Panulirus argus*	1	2	12	2	0	1	5	1	0	4	1	24	2	1	0	4	0	1	2	0				6	11	11	12	100	100	100	100	100	88
			Palinurus vulgaris																														85			100

[5] The numbers in the table are per cent values for the serological tests. Reaction between each antiserum and its homologous antigen is considered to be the 100 per cent reaction. The values for heterologous reactions are calculated on the basis of the homologous reaction. Values within 5 per cent of each other usually are considered to be of the same order of magnitude since the experimental error for these serological tests is ±2½ per cent. Comparative tests are read in vertical columns.

hemocyanins of the clawed lobster. *Homarus americanus,* illustrate this point. When antisera were made against the proteins of several species within a given systematic category surprisingly uniform orders of relationship with the proteins of species from other categories were established. The antisera against the hemocyanins of the five species in the Tribe Brachygnatha, for example, gave similar results with heterologous antigens from species outside of the Tribe.

Analyses of variance [3] were made of the data in table II. A separate analysis was made using each Tribe as a reference group. It is necessary to point out the different kinds of serological data which were used in the statistical analyses. In some experiments, for example in the anti-*Dromia* column, several antisera were prepared against the proteins of a single species. The heterologous reactions which were performed against each of these antisera, because of limitations of material, did not all involve the same number of tests or same kinds of species. As a result, the data obtained with each of these antisera were not completely comparable. An average order of relationship among the categories concerned was determined, however, from the four anti-*Dromia* sera. Every cross-reaction was counted as an individual observation for the Tribe which was represented.

In other experiments, the results of tests with antisera which were prepared against the proteins of several species from the same systematic category were averaged to give a single order of relationship for that category. The five kinds of anti-Brachygnatha sera illustrate this point. Each of the four antisera produced against the proteins of *Cancer pagurus* was counted as a separate experience in classification as was each antiserum produced against the proteins of *Cancer borealis, Callinectes sapidus, Pachygrapsus marmoratus* and *Libinia emarginata.* A total of eight antisera were involved in ascertaining the anti-Brachygnatha sequence. The order of relationship derived from a grouping of the data from these antisera is not strictly comparable to the order of relationship established using several antisera made against a single species. The greater the number of species in a given category, which are representated through antisera, the more is the likelihood of obtaining a correct sequence of relationship to that category. However, the prejudice which the results might show in favor of a single species against which several antisera have been made does not invalidate the order of relationship to the larger category in which the species is located.

The relative orders of relationship as established by the antisera in these tests are given in table III. The first Tribe of each list of Tribes is a reference group as determined by the antisera made against the hemocyanins of the organisms included in that particular systematic category. The averaged per cent relationship to the reference group is given in the table. The per cent values should not be construed as fixed values for intertribal relationships among decapod Crustacea, but rather the results obtained with the particular antisera and proteins involved. Additional tests would change the percentage values. The sequences of the categories would, however, remain relatively constant. The calculations indicated that there is a probability of less than 0.001 that any of these analyses represent a single population. The minimum significant differences between means, at a probability of 0.05, given in parentheses immediately following each reference category, do not permit a gradation of the Tribes any further than that given in table III. The consistency with which orders of relationship, based on reciprocal testing, verify one another, warrant, in my opinion further gradations. The statistical methods employed in the analysis are insufficient to detect the degrees of dif-

[3] Gratitude is expressed to Dr. Robert Sokal, Department of Entomology, University of Kansas, for his assistance in the mathematical analysis of the serological data.

TABLE III. *The relative orders of relationship among six tribes of decapod Crustacea as revealed by serological tests* [6]

TRIBES	%	TRIBES	%
Oxystomata (13)	100	Brachygnatha (22)	68
Brachygnatha	23	Oxystomata	49
Nephropsidea	10	Dromiacea	29
Dromiacea	8	Nephropsidea	19
Paguridea	5	Paguridea	3
Scyllaridea	2	Scyllaridea	3
Dromiacea (11)	100	Paguridea (19)	62
Oxystomata	13	Brachygnatha	11
Brachygnatha	12	Oxystomata	9
Nephropsidea	10	Nephropsidea	7
Scyllaridea	7	Dromiacea	3
Paguridea	6	Scyllaridea	1
Nephropsidea (8)	97	Scyllaridea (12)	97
Oxystomata	30	Nephropsidea	21
Brachygnatha	22	Dromiacea	18
Dromiacea	16	Oxystomata	15
Paguridea	13	Brachygnatha	12
Scyllaridea	10	Paguridea	7

[6] These relationships were determined from the values on table II. The fiirst Tribe given in each list is the reference group, and the other groups are indented in accordance with their relative relationship to it. The per cent values are the averages of all the reactions between the antisera to the proteins of species in the reference group and the proteins of the species in each of the other groups. The number in parentheses given immediately after each reference group is the minimum per cent difference, calculated at a probability of 0.05, which should exist between the averages of any two groups in that particular sequence, before the percentages can be considered to be significant. Indentations of the groups were determined on the basis of this minimum difference.

ference which are apparent from the empirical data.

DISCUSSION

The method of comparative serology makes possible a quantitative analysis of the chemical and structural correspondence among the hemocyanins in the sera of Crustacea. Relative orders of relationship among species can be established by this means. Any arrangement of organisms which is based on a single characteristic such as the hemocyanins of the sera has obvious shortcomings. Hemocyanins serve Crustacea in multiple ways and, because of this, are acceptable to all parts of the body. On such a basis the hemocyanins might be considered the most desirable single characteristic which could be used to designate these species. The best classifications among the living Crustacea, however, will be made by utilizing all the evidence available from all the various disciplines concerned with establishing animal relationships.

The statistical methods employed in the attempt to ascribe numbers to a natural system emphasize the limitations of both the statistics and the serological method. The per cent relationships, established in any series of heterologous reactions to a given antiserum, are a true indication of the discriminating capacity for that particular antiserum. When the values for several antisera, each with its own specificity, are averaged together the resultant values may still show central tendencies but the trueness of the taxonomic information can rightfully be questioned, in view of the fact that non-equivalent sources (i.e., the different antisera) were used to arrive at a value for a systematic category. The minimum significant differences between categories which were

determined for each order of relationship (table III) are useful but do not necessarily indicate a biological reality.

A cogent criticism [4] of the serological data as it is presented in this paper is that more or less exact relationships of several decapod groups are indicated on the basis of admittedly inadequate comparisons of the sera of only 2 of the 84 known scyllarids, 2 of the 32 homarids, 4 of the 555 pagurids, 1 of the 156 dromiids, 1 of the 480 oxystomes, 5 of the 2,830 brachyrhynchs, and 2 of the 723 oxyrhynchs. With so few examples, no assumptions can be made that we are dealing with average species in the groups, or even with closely related or extreme forms of two adjacent groups. Following existing classifications it is possible for serologically similar species to belong to two groups, and conversely, for rather dissimilar species to be found in the same group.

Despite the more obvious shortcomings, the serological method still provides a useful quantitative measure of the degree of correspondence among the hemocyanins of Crustacea. Degrees of relationship to the homologous antigen are established with each antiserum. Every such grouping is objective in the sense that human interpretation is at a minimum, and natural in that the rabbit provides data on the biochemcial similarity of antigens without prejudice, in the ordinary sense of the word. No implication is intended here to establish the rabbit as the infallible arbiter of animal relationship. Variations in "interpretation," and even errors are possible in the varying responses of rabbits to antigens. It is remarkable that the results are as consistent as they are.

A unique advantage in serological comparisons among the corresponding proteins of species is that each antiserum provides a new point of view. Several antisera, produced against the proteins of one species, may differ widely in their specificities and strengths of reaction. Antisera may be "lumpers" or "splitters" with regard to their discriminating capacity among the members of a series of heterologous antigens. Each antiserum, because of its particular characteristics, provides useful information on the relative similarities and differences among the various antigens which are tested. Through the responses of the rabbits, it is possible to move from locus to locus of a group such as the decapod Crustacea, and view the situation anew with each antiserum. No other discipline, thus far applied to the classification of animals, permits this. The number of arrangements is limited only by the number of antisera available.

It should be pointed out here that the sequences established in table III are not linear. At least three dimensions are required to express more correctly the relationships. I have attempted to arrange the six Tribes of decapod Crustacea in a three dimensional graph (fig. 2). The subtribes Oxyrhyncha and Brachyrhyncha of the Tribe Brachygnatha were considered separately in the construction of the model. Each of the seven groups was represented as a point in space. The "distance" between any two points was calculated in accordance with the suggestions of Boyden (1926); by subtracting the per cent of heterologous reaction from 100 the relative relationship value is converted into a relative "distance" value. In this manner, organisms which have a large per cent of relationship will have relatively short "distances" between them. For example, table II shows that the proteins of *Cancer borealis* had a reaction which was 70 per cent of the homologous reaction between the proteins of *Cancer pagurus* and the anti-*Cancer pagurus* serum. The relative "distance" between these two species, for this antiserum, is 30. On the other hand, the proteins of *Pagurus prideauxi* had a reaction of only 11 per cent of the homologous reaction. The relative "distance" value between the two species, *Cancer pagurus* and *Pagurus prideauxi*, for this antiserum, is 89. All

[4] Suggested in personal communications with Dr. Fenner Chase, U. S. National Museum, Washington, D. C.

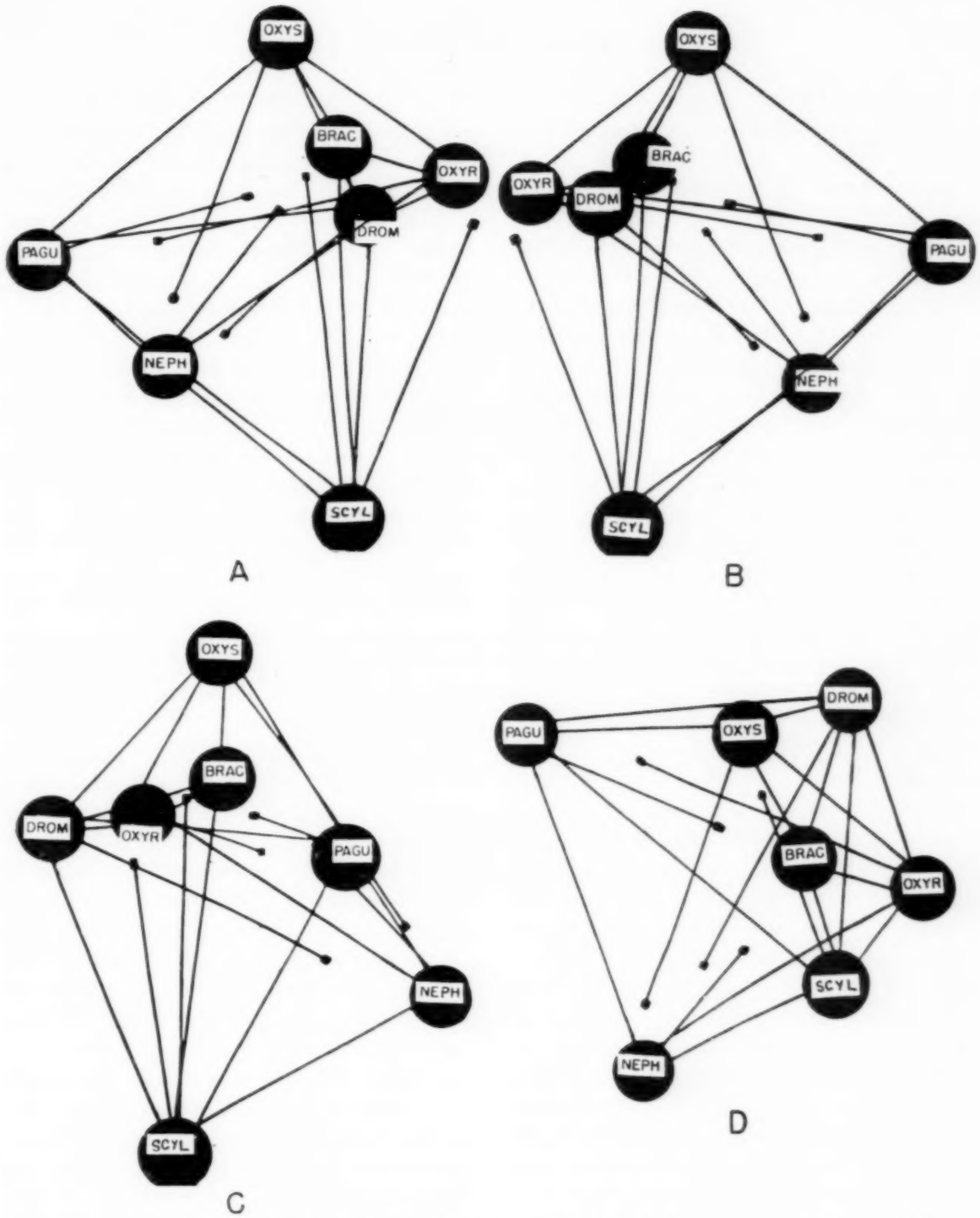

FIG. 2. A three-dimensional representation of the relationships among seven groups of decapod Crustacea. Four views of the same model are presented to more clearly show the interrelationships. Views A, B, and C are lateral; view D is polar looking down through the model toward the scyllarid locus. The orientation of the model, with the Scyllaridea at the bottom, was chosen to conform with the phylogeny given in figure 3. Black blocks have been placed on the rods which do not fit to emphasize their location. The tendency toward fitness is exhibited among these rods in that long rods extend between more distantly located loci, and short rods extend between closely located loci. The four groups of brachyuran crabs form a cluster of loci. The locus for the clawed lobster (Nephrosidea) is closer to this cluster than is the locus for the hermit crabs (Paguridea). The locus for the spiny lobsters (Scyllaridea) is most distantly situated from the Brachyura.

Brach = Brachyrhyncha
Oxyr = Oxyrhyncha
Oxys = Oxystomata
Drom = Dromiacea
Neph = Nephropsidea
Pagu = Paguridea
Scyl = Scyllaridea

of the relationship values between any two groups (table II) were averaged in order to obtain a single "distance" value between them. It is apparent from the serological data (table II) that the differences among close relatives are disproportionately emphasized as a consequence of the specificity of the various antisera. A 10 per cent variation in the reaction shown by several species of Brachyrhyncha to an anti-Brachyrhyncha serum represents smaller differences among these species than a 10 per cent variation in reaction between a species from the Astacura and a species from the Anomura. On this basis, an ordinary linear scale to mark off inter-group distances is less suitable than is a logarithmic scale. A logarithmic ruler was constructed and used to mark off the "distances" between the groups of organisms. In this manner it was possible to emphasize the similarities among close relatives and the differences among more distantly related groups. The various loci were oriented with respect to one another so as to present the best fit. As the three-dimensional classification is represented in figure 2, it can be seen that several of the "distance" bars are too short. The tendency toward fitness is there, however, even among the ill-fitting "distance" bars, in that the shorter bars extend between loci which are closer together in space, and the longer bars extend between the more distantly spaced loci. The particular orientation shown with the Scyllaridea at the base of the model was chosen because it most nearly conformed to generally accepted paleontological evidence. Even a three-dimensionar representation of relationships becomes inadequate as the number of species, or in our case groups of species, exceed five or six (see fig. 2). If the "distances" are represented by stiff rods, the model fails as soon as rigidity is obtained because all of the reciprocal "distances" do not fit. Flexible rods permit the fitting of a few more "distances" into the various loci, but, again, limitations inherent in a rigid system would limit the number of rods which fit. In a flexible-rod model, moreover, the spatial arrangement becomes inaccurate because distantly related species come to lie close to each other in contradiction to both serological and accepted morphological data. The failure of all the results to fit perfectly into a three-dimensional model does not invalidate the serological data.

The distance-values which are used here to indicate relationships are, in a sense, artifacts. They attempt to measure two dimensions simultaneously, which is an obvious impossibility. The first of these dimensions is geologic time. The species undoubtedly reached their recent stage of development at different times. Rabbits produce antisera against the antigens which are presented to them so that any classification derived from these antisera measures the serological correspondence as it exists now, the second dimension. Genera, such as those in the tribe Brachygnatha, which seem to be undergoing an eruptive type of evolution at present are compared on the same standard as are the Scyllaridea which today are a relic group. The rods of the model serve no other purpose than to separate the various loci by a serologically determined "distance," and hence, should not be construed as representing possible lines of derivation.

Figure 3 is a representation of the phylogeny of the decapod Crustacea. The figure is an attempt to merge the original representation of Ortmann (1901) with the supplementary modifications of Borradaile (1907) and Calman (1909). The correlation between morphological classification (table II) and paleontological derivation on the one hand and the serological arrangements of the Crustacea on the other is good. Variations in the reactions of individual antisera in the qualitative differentiation of heterologous antigens were occasionally obtained, but most of these were within the limits of error of the experimental method. The most questionable of the serological tests are the comparisons between the proteins of the

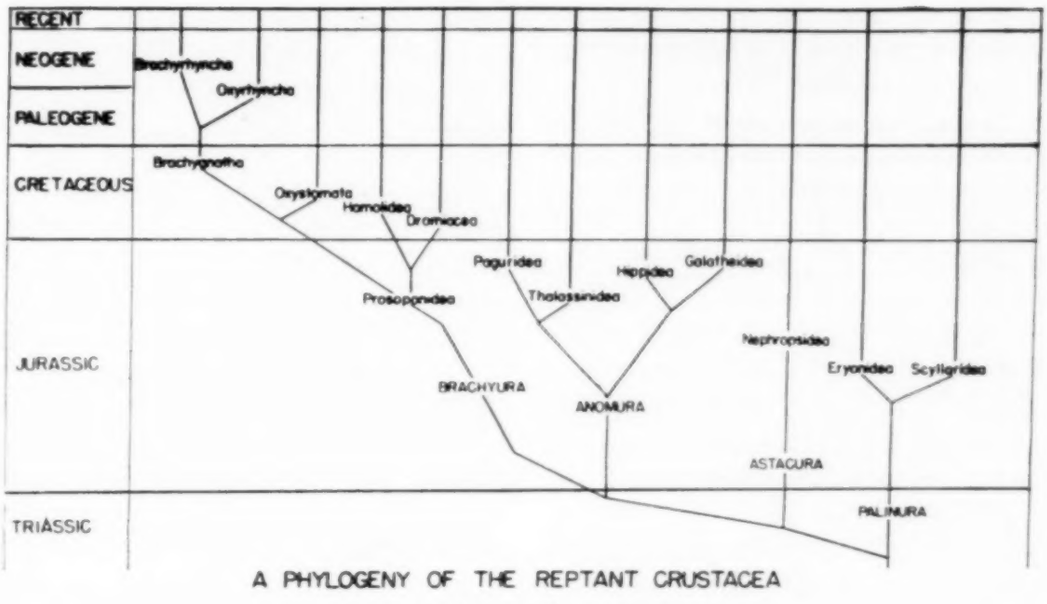

FIG. 3. A representation of a possible phylogeny of the decapod Crustacea compiled and modified from Ortmann (1901), Borradaile (1907) and Calman (1909). The positions of the various words, with respect to the geological periods given on the left of the figure, indicate the approximate time when the particular group of animals appeared in the fossil record. The connections among the four major lines have no basis in fact from the fossil record and indicate, merely, the possibility that the decapod Crustacea were derived from a common ancestral stock.

brachyuran *Dromia vulgaris* and the scyllarid *Panulirus argus*. From the data in table II it can be observed that one of the anti-*Dromia vulgaris* sera gave an unusually large reaction with the proteins of *Panulirus argus*. Similarly, one of the several anti-*Panulirus argus* sera gave an unusually large reaction with the proteins of *Dromia vulgaris*. The suspicion arose that lipoproteins, or perhaps Forsmann-like antigens might be influencing the reactions. In order to test this possibility, antisera made against representative species of each of the seven categories were selected and were absorbed three times with washed red blood cells of sheep to remove any antibodies to Forsmann-like antigens. In addition, in order to remove any lipid materials which were present, the homologous antigens to each of these antisera were extracted at 0° C. with a volume of acetone equal to the volume of the antigen solution. The hemocyanins were completely precipitated by this concentration of acetone. After decanting the acetone from the precipitated protein, and removing the last of the acetone *in vacuo*, the precipitates were dissolved in buffered saline. This procedure was performed twice on each sample of antigen. The lipid-extracted antigens were tested against absorbed and unabsorbed samples of the same antiserum. No modifications of the orders of relationship as previously established by these antisera were noted as a result of this series of tests. It would seem, therefore, that neither Forsmann-like antigens nor lipoproteins have influenced the serological relationships as reported in this paper. No adequate explanation is advanced to explain the unusual *Dromia-Panulirus* relationship which was indicated above. Speculation as to the occurrence of parallel evolution of the proteins of these two species is not warranted on the basis of this evidence. The possibility that *Panulirus* and *Dromia* represent one evolutionary line, and that all of the other decapods represent another line, is likewise not indicated.

From the points of view of the anti-

Paguridea rabbit sera (table II) the closest relatives of the pagurid crabs are the species in the subtribe Brachyrhyncha. The serological evidence does not imply that the Anomura were the ancestors of the Brachyuria, nor is the converse of this implied.

The serological data indicate that the Scyllaridea are distinct from all of the other Crustacea which were tested. The fact that there is more serological correspondence among the proteins of the Brachyura, Anomura and the Nephropsidea than between the proteins of the Nephropsidea and the Scyllaridea may be surprising to some students of these arthropods, but it lends impressive support to Ortmann's (1907) original representation of the evolution of the Decapoda.

A phylogeny traces the broad outlines of racial descent of a major animal group. The object of classification is not to express the phylogeny of a group but rather to arrange organisms in accordance with their similarities and differences (Boyden, 1947). Effective and useful classifications can be constructed in the absence of a paleontological record. A phylogenetic tree constructed exclusively from living forms, and without recourse to the paleontological record, is an obvious absurdity. The relative primitiveness of a form, can be established, with certainty, only from the paleontological evidence. A classification which is in harmony with the phylogenetic record, where the latter is good, approaches a "natural classification." As taxonomic data on a given group of animals continues to accumulate, the likelihood of obtaining a natural system of classification improves.

Summary

The sera of seventeen species of decapod Crustacea representing the Tribes Brachygnatha, Oxystomata, Dromiacea, Paguridea, Nephropsidea and Scyllaridea were compared serologically. The sera of species from each group were used to produce precipitins in the rabbit. Appropriate homologous and heterologous serological tests were run and the qualitative differences among the crustacean sera were quantitatively measured by using a photoelectric turbidimeter on the white precipitates which developed. The results of the serological comparisons were compared with the classification of the Tribes based on comparative morphology and the phylogeny of the decapod Crustacea based on paleontological evidence. In general the correlation was close. The Nephropsidea had no closer relationship to the Scyllaridea than did the Brachygnatha, or Oxystomata. The Oxystomata had greater serological correspondence to the Brachygnatha than did the Dromiacea. The Paguridea exhibited a closer relationship to the Brachygnatha than to any other of the groups tested.

Each antiserum established, in accordance with its specificity, an order of relationship to the test antigens. The orders of relationship established by antisera made against the serum proteins of the same species were almost always uniform. Antisera made against the serum proteins of closely related species show a high degree of uniformity as concerns their reactions with the proteins of species from distantly related groups. The constancy of the phenomenon of quantitative serological specificity as demonstrated by the antisera to the hemocyanins of these Crustacea supports the contention that serum proteins are the results of conservative evolutionary processes.

Where data from morphological and paleontological studies are inadequate for the purpose of establishing degrees of relationship among recent groups of animals, the serological method can provide a measure of the relative similarities and differences among organisms and contribute supplementary information on their taxonomy.

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A PARTIAL BREAKDOWN OF TEMPORAL AND ECOLOGICAL ISOLATION BETWEEN *CHORISTONEURA* SPECIES (LEPIDOPTERA: TORTRICIDAE)[1]

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Received January 5, 1954

INTRODUCTION

The spruce and jack-pine budworms have for some years been regarded as distinct, but closely related, species in the genus *Choristoneura,* and the latter, *C. pinus* Free., has recently been formally separated from the former, *C. fumiferana* (Clem.), by Freeman (1953). Because they will hybridize with comparative ease in captivity, yet show no evidence that cross-mating occurs in nature, the factors that ensure their integrity have been studied over a number of years and were recently summarized (Smith, 1953).

Numerous larger or smaller segments of the 2 species are isolated geographically owing to the different spatial distributions of their respective host trees. Where populations have common perimeters, crossing is seemingly prohibited by a number of intrinsic components that jointly confer upon the species absolute reproductive isolation. These have been found to include ecological, temporal, and sexual barriers. The most obvious of these is the temporal factor, for, so far as was known up until April, 1952, the mating season of *fumiferana* is over before *pinus* adults appear on the wing. This seasonal isolation results from the later spring emergence of overwintering *pinus* larvae (phenological isolation) combined with a slower rate of subsequent development (ontogenetic isolation).

In the course of a survey of jack-pine budworm, an unusual and interesting situation was encountered near Blue and Indian Lakes on May 11, 1952. These lakes are situated about 10 road-miles NW. of Vermilion Bay in the Kenora District of Northwestern Ontario. Examination of jack pine male cones revealed the presence in them of late second- and early third-instar budworm larvae at a time when larvae in adjacent balsam fir foliage were in comparable, although slightly advanced, stages. Previous experience in an area of common occurrence during 1948 had set at 11 days the differential in resumption of activity, with *fumiferana* larvae having appeared on May 10 and *pinus* larvae on May 21 in that year.

The absence of a marked developmental difference between larvae on jack pine (Pj) and balsam fir (Bf) in 1952 suggested 2 possible explanations, both of which were open to test: (a) the host discrimination usually attributed to larvae of the 2 species had broken down, at least in the case of 1 of them, or (b) the usual difference in time of spring emergence had failed to materialize. The fact that the weather during late April and early May had been unseasonably hot, with daily temperatures frequently running up into the eighties—see Rose and Blais (1954) for details, favoured the latter alternative. Plans were accordingly laid to check this inference and, should it prove correct, to be on hand throughout the overall flight period to assess the contributions to reproductive isolation made by the temporal, ecological, and sexual components.

EFFICACY OF PHENOLOGICAL AND ECOLOGICAL ISOLATION

About 120 larvae were collected from Pj staminate cones and some 250 from

[1] Contribution number 127, Division of Forest Biology, Science Service, Department of Agriculture, Ottawa.

Bf foliage. Each collection was split into 2 equal lots to provide 4 samples, which were then set up for rearing as follows: presumed *fumiferana* larvae on (i) Bf and (ii) Pj; presumed *pinus* larvae on (iii) Pj and (iv) Bf. Mortality in rearing, carried out at Sault Ste. Marie, proved to be extensive, partly owing to parasitism but mainly because local Pj trees were so far retarded compared with those in the Kenora District that appropriate staminate cones and young foliage were not available as a continuing source of food. Mortality in lot (ii) was later checked by returning it to a diet of Bf. The results nevertheless sufficed to show that the difference in emergence time had failed. A breakdown in host specificity, however, as judged from these rearings, apparently involved only *C. fumiferana*. Limited collections, subsequently provided, confirmed the former conclusion. They also implied that, had the host preference actually failed, for 1 or both species, then, by the fourth instar the larvae must either have sorted themselves out according to host or those on the "wrong" host must have succumbed. Evidence from mass collections made around pupation time only partially substantiates this implication (see later) but is quite compatible with the interpretation based on host transference results.

These preliminary tests, together with the developmental status of the collections from time to time supplied pointed with virtual certainty to the realization of the promised overlap of adult moths. The tentative plans to establish an observation plot at Blue Lake were therefore set in operation.

Techniques

Description of site

The selected plot, situated on a sandy slope having a SW. exposure, lent itself admirably to the observations planned. It consists of a checker-board mixture of open-grown Bf and Pj trees between 15 and 25 feet in height, along with a few black and white spruces and a fair number of poplars and white birches. In contrast to the plots established in 1948 and 1949 at the Wabigoon River and McIntosh Road, the Pj trees at Blue Lake, being open-grown, were bushy, with branches down almost to ground level, so that they were readily accessible for close examination. One further feature that ameliorated the task of adequate sampling was the heavy crop of staminate cones that cloaked even the lowest-hanging branches of the Pj trees, for such cones are the preferred feeding and subsequent pupation sites.

Methods of Sampling

Four lots of trees, 2 of Bf and 2 of Pj, were pre-selected for observation, 1 of each being reserved for adult population density assessment, the others for recording mating frequency and time.

To secure as complete a picture as possible, daily samplings were obviously preferable and so far as possible these should have proceeded daily, from the beginning to the end of mating activity, throughout the flight period of both species. Unfortunately no assistance was available, so that all sampling and other observations had to be carried out unaided. This was compensated for, however, in that any variability attributable to personal differences in unconscious bias was eliminated. The isolated location of the plot necessitated daily travel from the Cedar Lake Field Station over some 75 miles of poorly conditioned roads. Combined with the fact that mating activity is apt to occupy the period from midday to midnight, or even later, and to extend over more than a month, this rendered it impracticable to compile records of mating time throughout every day. A compromise was reached by alternating "long" and "short" days. Long days were initiated only following the appearance of adults of both species and lasted from 1415 hrs. CST until mating activity was concluded for the day. On short days and during that part of the flight period when only 1 species was

active, observation commenced at 1200 hrs. and ended each day after cage emergence data and population density information had been recorded, usually by 1630 hrs.

Routine daily observation started June 29 and continued through to July 25. Evening mating activity was recorded July 6 and 7 and thereafter on long days through until July 21; on the remaining short days usually only afternoon records were compiled.

Adult population census: A daily census by species and sex was taken on the basis of the number of adults that could be captured, sexed, and, in the case of certain of the females, transferred to vials during a 30-minute period. Spent females were discarded after being recorded as "mated"; unspent females were retained pending determination of their nuptial status. When adults of only 1 species were present, counts were taken between 1430 and 1500 hrs.; when both were on hand, from 1415 to 1445 hrs. for *fumiferana* moths and from 1515 to 1545 hrs. for *pinus* adults, the intervening half-hour being devoted to sampling for mating frequency. All moths were readily captured by dislodging them into an 8-ounce jelly jar secured to the end of a 5-foot pole.

Sampling for mating activity: When adults of both species were present in the field, observation of mating activity was carried out every hour for a period of 30 minutes, Bf trees being under examination during the 15 minutes preceding the hour and Pj trees during the succeeding 15 minutes. Copulating pairs were recorded as to time and the species involved. After dusk, observations were made with the aid of a 5-cell flashlight. The strength of the beam provided adequate coverage of the relatively small trees and, in fact, allowed detection of mating pairs quite as readily as during daylight, because attention could be more localized and was less distracted by disturbing events outside the illuminated area.

Observations

Ecological Isolation

Larval: In order to supplement the estimates of the comparative phenology of the 2 species as determined by daily population sampling, mass collections were made from Bf and Pj trees located just outside the prescribed plot site. These provided, in addition, evidence concerning the efficacy of ecological isolation during the larval stage, *i.e.*, that attributable to differences in host-tree preferences. Although no *pinus* adults were obtained from the collections secured from Bf, about 3.2% of the adults derived from Pj trees proved to be *fumiferana* (table 2). The deferred eclosion of these (table 3) suggests in the strongest terms that they had in fact matured on a diet of Pj and effectively disposes of the alternative possibility of migration during late larval life from Bf to Pj.

Adult: So long as unmated adults remain confined to strictly pre-selected host trees, no hybrid unions are possible, and ecological isolation is fully effective whether or not a lapse in temporal isolation materializes. Since, however, a small percentage of the *fumiferana* population was found to have matured on Pj trees, with consequent retardation in emergence, some failure of the limitation imposed by different ecological requirements was to be expected.

Throughout the course of the routine observations of mating activity, records of the association of moth species with tree species were taken for *fumiferana* and *pinus* adults. The breakdown of these as mating males and females and as mated (i.e., partially or completely spent) and unmated females on the 2 host trees is given in table 1.

From the first day on which they appeared, and hence even before mating, males were observed to be active in flying; females, on the other hand, were largely quiescent, especially when obviously still retaining the majority, if not all, of their original complement of eggs.

TABLE 1. *Ecological isolation of adults*

Host	Number of *C. fumiferana* ♂♂	Pairs	Mated ♀♀	Unmated ♀♀	Number of *C. pinus* ♂♂	Pairs	Mated ♀♀	Unmated ♀♀
Bf	289	204	190	79	7	(1)	2	0
Pj	31	1	11	1	113	80	62	54
% failure	9.7	0.05	5.5	1.2	5.8	(1.2)	3.1	0.0

Support is therefore afforded for Wellington's (1948) conclusions regarding the handicap imposed on female flight by the weight of their contained eggs. The seemingly contrary evidence provided by the occurrence of an unmated *fumiferana* female on a Pj tree and single mating pairs on each of the "wrong" trees calls for special comment. The presence of the *pinus* pair on Bf may almost certainly be discounted, for the tree in question was situated directly under a larger, overhanging Pj tree, from which the pair had doubtless been dislodged. The 2 *fumiferana* females, 1 unmated and the other mating, occurred on relatively isolated Pj trees and thus cannot logically be regarded as accidental transpositions. It seems more reasonable to conclude, especially in view of the presence of *C. fumiferana* individuals in the mass collection made from Pj, that the 2 females had emerged *in situ*, and that the copulating male had been attracted to its mate while flying nearby. If such were the case, it might then be assumed that the ecological isolation stemming from differences in food preference plays only a minor, subsidiary role in reproductive isolation. That this conclusion is valid is made highly probable by the freedom with which males indulge in flight, for this renders up to 50% of each species unencumbered by confinement to a particular host tree. Whether the 9.7% and 5.8% of the males that were misplaced comprise largely mated individuals, as is so with females, could not be determined. But that some were unmated follows from the fact that a few males were so situated before their corresponding females entered the adult population.

Temporal Isolation

Mean emergence date and extent of failure of temporal isolation

1. *Evidence from mass collections:* Phenological and developmental differences between sympatric populations that restrict the proportions of 2 species attaining maturity synchronously constitute a temporal, or seasonal, barrier to gene exchange. The determination of the weighted percentages of the 2 populations that escaped this limitation is a prerequisite to assessing the efficacy of sexual isolation. Since only indirect measurements of combined phenological and developmental differences are determinable by routine sampling of populations of adults, mass collections were made before maturity from the inhabitants of neighbouring Bf and Pj trees. A total of 320 larvae, prepupae, pupae, and empty pupal cases (numbering 5 and determined to be males) were taken from Bf and placed with food in a cage on June 28 and 29. A total of 250 were collected from Pj, along with the terminal on which each occurred, and similarly caged on June 29 and 30 and again on July 5. A comparison of the percentages of larvae in the Bf and Pj collections made on the 29th, namely, 10 and 85 respectively, readily establishes that the *fumiferana* population was considerably advanced relative to that of the *pinus* species. A measurement of this difference is readily obtained by comparing the mean dates at which adults of the 2 sexes of the 2 species eclosed.

The numbers of adults that emerged by 1600 hrs. daily, both absolute and on a percentage basis, are given by species and sex in table 2 (fig. 1). As is to be

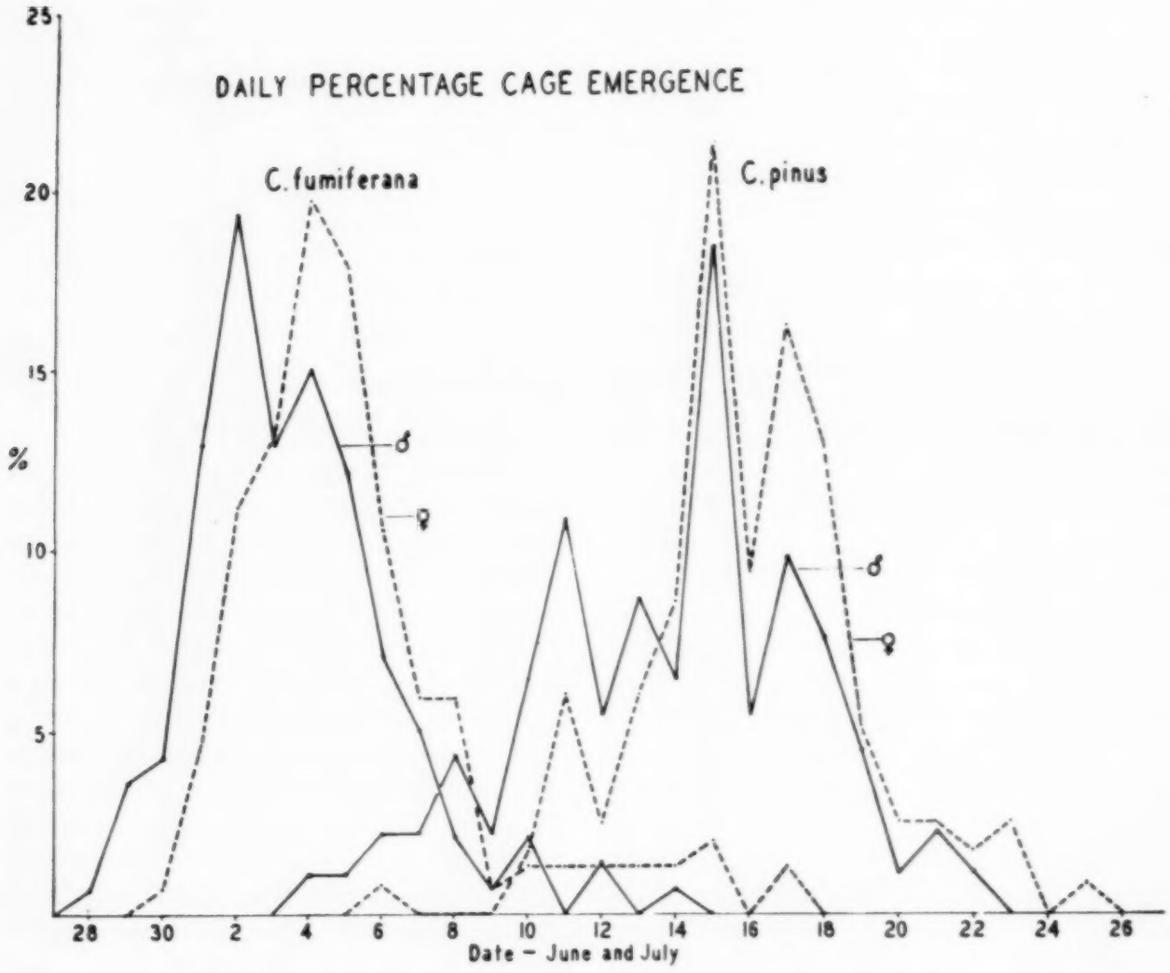

FIG. 1. Daily percentage frequency distributions of emergence by sexes from mass collections of *C. fumiferana* and *C. pinus*.

expected from determinations of the relative development rates established in laboratory rearings (Smith, 1953), males of both species eclosed in advance of their females and *fumiferana* as a whole considerably predated *pinus*. These records show that some 54% male and 30% female *fumiferana* eclosion was accomplished before the first *pinus* male emerged. At the other end of the eclosion period, about 16% and 28% of the *pinus* males and females respectively failed to emerge until after the last *fumiferana* female came off. The percentage of adults of the 2 species that eclosed on the same days, therefore, approximates 68, or two-thirds of the total cage population. This value does not, of course, set a corresponding limit to the proportions of the 2 species that would escape temporal isolation in the field, for it fails to take into account the intraspecific sex differential in emergence. Clearly, if it be assumed that females mate on the day of eclosion, as they probably do, only 30 of the 54% *fumiferana* males that have eclosed will have mated by the time *pinus* males enter the population. Similarly an excess 12% of *pinus* males will remain unmated pending the emergence of *pinus* females. Consequently, the extent of breakdown of temporal isolation approximates $70 + 70 + 72 + 72/4$, or 71%. It will be shown later that, owing to the disproportion in which the sexes and species occur on different days, the incidence of potential hybridization is considerably less than half of the temporal isolation breakdown percentage, i.e., considerably less than 35.5%.

TABLE 2. *Absolute and percentage daily cage emergence by species and sex*

Date	Adults from 'Bf' cage		Adults from 'Pj' cage			
	fumi. ♂♂	*fumi.* ♀♀	*pinus* ♂♂	*pinus* ♀♀	*fumi.* ♂♂	*fumi.* ♀♀
52-06-28	1 (0.7)					
-29	5 (3.6)					
-30	6 (4.3)	1 (0.7)				
-07-01	18 (12.9)	7 (4.6)				
-02	27 (19.3)	17 (11.2)				
-03	18 (12.9)	20 (13.2)				
-04	21 (15.0)	30 (19.7)	1 (1.1)			
-05	17 (12.1)	37 (17.8)	1 (1.1)			
-06	10 (7.1)	16 (10.5)	2 (2.2)	1 (0.8)		
-07	7 (5.0)	9 (5.9)	2 (2.2)	0		
-08	3 (2.1)	9 (5.9)	4 (4.3)	0		1
-09	1 (0.7)	1 (0.7)	2 (2.2)	0		
-10	3 (2.1)	2 (1.3)	6 (6.4)	2 (1.7)		
-11	0	2 (1.3)	10 (10.8)	7 (6.0)	1	
-12	2 (1.4)	2 (1.3)	5 (5.4)	3 (2.5)		
-13	0	2 (1.3)	8 (8.6)	7 (6.0)		
-14	1 (0.7)	2 (1.3)	6 (6.4)	10 (8.5)	1	1
-15		3 (2.0)	17 (18.3)	25 (21.2)		2
-16		0	5 (5.4)	11 (9.3)		1
-17		2 (1.3)	9 (9.7)	19 (16.1)		
-18			7 (7.5)	15 (12.7)		
-19			4 (4.4)	6 (5.1)		
-20			1 (1.1)	3 (2.5)		
-21			2 (2.2)	3 (2.5)		
-22			1 (1.1)	2 (1.7)		
-23				3 (2.5)		
-24				0		
-25				1 (0.8)		
Total	140	152	93	118	2 +	5 (=3.2%)

The records compiled in table 2 provide the mean emergence figures by species and sex that are listed in table 3. It will be seen that *fumiferana*, as a whole, reached maturity 10 to 11 days earlier than *pinus*.

As has been mentioned previously, only *fumiferana* moths were obtained from the mass collections made from Bf trees, whereas 2 *fumiferana* males and 5 *fumiferana* females occurred along with the 211 *pinus* adults that were derived from the Pj mass collections. The close agreement between the average dates on which these "misplaced" *fumiferana* individuals eclosed and those for the 2 sexes of *pinus* (table 3) is compelling evidence that the food preference broke down, probably at

TABLE 3. *Mean emergence date by species and sex: cage records taken daily at 1600 hrs. CST*

Species	Sex	Mean	*pinus-fumiferana*
C. fumiferana	♂	July 4, 0524 hrs.	10 d. 4 h. 6 m.
C. fumiferana	♀	July 5, 2130 hrs.	10 d. 22 h. 30 m.
C. pinus	♂	July 14, 0930 hrs.	
C. pinus	♀	July 16, 2000 hrs.	
C. fumiferana (ex Pj)	♂	July 12, 1712 hrs.	1 d. 16 h. 18 m.
C. fumiferana (ex Pj)	♀	July 14, 0624 hrs.	2 d. 13 h. 36 m.

the time of emergence from overwintering, and that their Pj diet reduced their normally enhanced rate of development.

2. Evidence from daily population census: The counts from the 30-minute daily samples of *fumiferana* collected between June 29 and July 23 and those for the *pinus* adults collected between July 6 and 25 are, to conserve space, not given in tabular form. It is possible, however, to extract them from tables 4 and 9. The total numbers of *fumiferana* males and females taken were 371 and 351 respectively, of which 164 were engaged in mating; those for *pinus* were 122 and 105, of which 18 were *in copula*. As is suggested by their being at variance with the results obtained from mass collections (table 2), the excess of males in the field samples of both species is most likely a consequence of attention being drawn disproportionately to males by their greater activity, supplemented by the tendency of females to remain at the higher levels at which they mature.

In order to compute the extent of breakdown in temporal isolation, it was necessary to determine the nuptial status of the females. This could not be done directly for non-mating males. The status of mating and spent females is self-evident. Gravid females, confined to the appropriate foliage, may be typed with confidence as mated or not on the strength of the appearance of any egg clusters subsequently deposited (if none is produced,

TABLE 4. *Daily population densities and nuptial status by species and sex: hourly basis*

Date	Numbers of *Choristoneura fumiferana*				Numbers of *Choristoneura pinus*			
	Mated ♂♂	Virgin ♂♂	Mated ♀♀	Virgin ♀♀	Mated ♂♂	Virgin ♂♂	Mated ♀♀	Virgin ♀♀
June 29	1	8	1	2				
30	3	18	3	8				
July 1	8	12	8	6				
2	16	18	16	12				
3	10	30	10	12				
4	18	36	18	14				
5	24	12	24	12				
6	31	4	31	8	0	2		
7	29	10	29	6	0	6		
8	32	22	32	8	0	8	0	2
9	25	6	25	8	2	4	2	2
10	26	32	26	10	2	8	2	4
11	23	14	23	8	4	8	4	2
12	29	16	29	4	2	8	2	2
13	36	16	36	10	3	8	3	6
14	40	0	48	10	22	4	22	12
15	20	0	28	6	19	16	19	12
16	16	0	22	4	16	28	16	8
17	8	0	14	6	13	8	13	4
18	7	0	19	2	12	0	14	4
19	4	0	12	2	8	0	8	4
20			6	0	4	0	4	4
21			0	0	4	0	6	0
22			0	0	6	2	6	2
23			2	0	2	0	6	0
24					4	2	4	0
25							2	0
Total	406	254	462	158	123	112	133	68

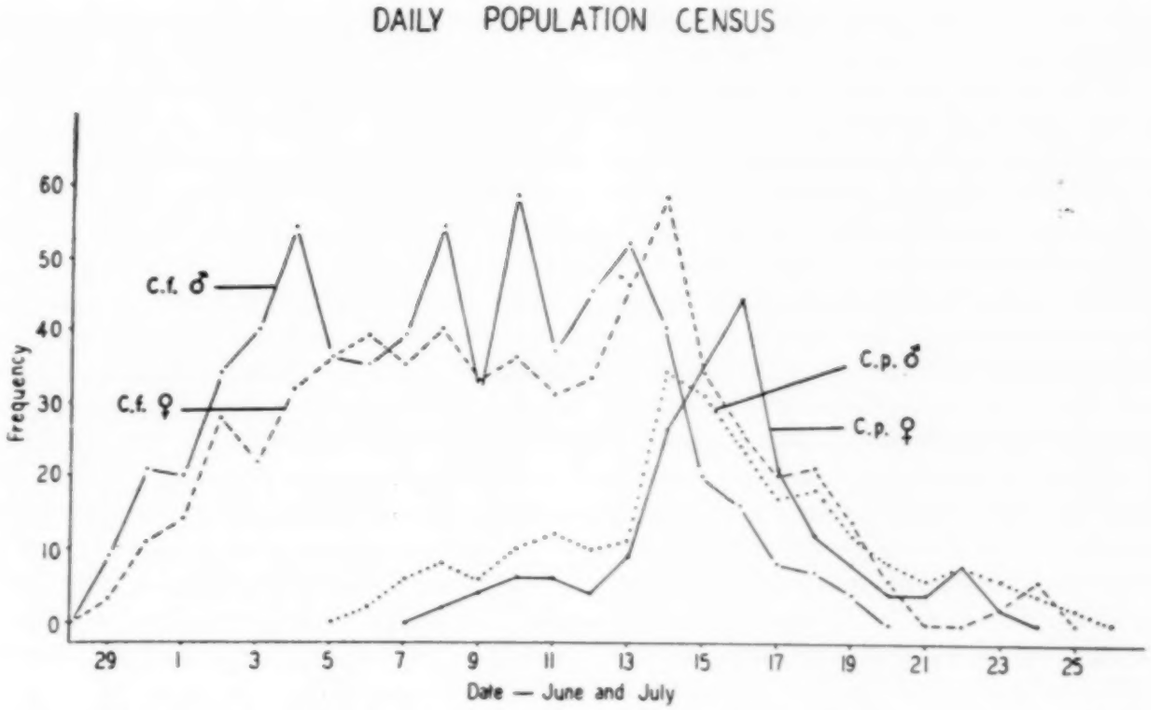

FIG. 2. Daily numbers of adults of *C. fumiferana* and *C. pinus* by sex collected from the field (see text).

the female may safely be judged as virgin). Typically formed egg clusters denote that the female had mated; small, irregular, scattered masses establish her as virgin. Determined in this way and converted to an hourly basis (see later), the numbers of unmated (including those *in copula*) and mated females and, by inference, of males occurring daily are given in table 4.

The raw data assembled in table 4 unfortunately provide only the grossest estimate of the actual population trends within the 2 species, primarily no doubt owing to the increasing limitation imposed on handling as the numbers of adults approached maxima. The influence of this limitation is obvious in figure 2, where the curves for *fumiferana* males and females flatten out and become erratic about July 4, instead of following the trends established earlier. It also distorts the relative frequencies of the 2 species, since *fumiferana* proved to be much more dominant and the pressure of numbers correspondingly more effective. On the other hand, *fumiferana* adults are more readily detected than are *pinus* adults. The colour of the latter moths, which is doubtless a selective adaptation, renders them easily confused with the dried, rusty-coloured staminate cones, near which they are usually found; whereas *fumiferana* moths, particularly when engaged in mating, usually hang from the abaxial surface of the pinnately-arranged needles of the horizontal branchlets, and consequently can be seen and dislodged into the collecting jar with greater ease. In the case of mating moths, since no time is consumed in sexing or in transferring females to vials to check their nuptial status, and since each pair contributes a value of 2 to the actual count, the relative numbers of *fumiferana* adults appear to be higher than those of the later mating *pinus* species (table 9). Each pair is therefore computed as only one-half mated male and one-half mated female for purposes of assessing population density. Thus in table 4, where, to avoid fractions, the daily values have been converted to an hourly basis by doubling the frequencies of non-mating moths, a mating pair contributes unity to each sex. The actual

daily numbers of such pairs appear in table 9.

To overcome the inherent deficiencies in the sampling technique, the distributions of females of each species collected *in copula* or tested and found to be virgin have been fitted to curves plotted on arithmetic probability graph paper and derived from the cumulative percentages of females that emerged in the mass collection cages. In so doing, it is assumed that the daily frequencies of mating and unmated females would be comparable to those obtaining in cage emergences were the latter not subject to errors in sampling. In this way the actual numbers, not the percentages (since these are distorted throughout by the deficiencies at and around peak emergence), of such *fumiferana* females caught in the periods June 29 to July 4 and July 11 to 24 have been fitted to the corresponding parts of the theoretical probability curve, and frequencies have been calculated for the intervening days. Since sampling deficiencies affect both mated and unmated individuals equally, it is then possible, on the basis of the virgin-female distribution, to construct theoretical curves for both the total females (mated + unmated + mating) and the total males. This results in an increase in the sizes of the 2 samples from 660 for males and 620 for females to roughly 1475 for each. Comparable but lesser increases result for *pinus*. Finally, the values on which the 2 virgin male curves were constructed are the differences between the daily numbers of total

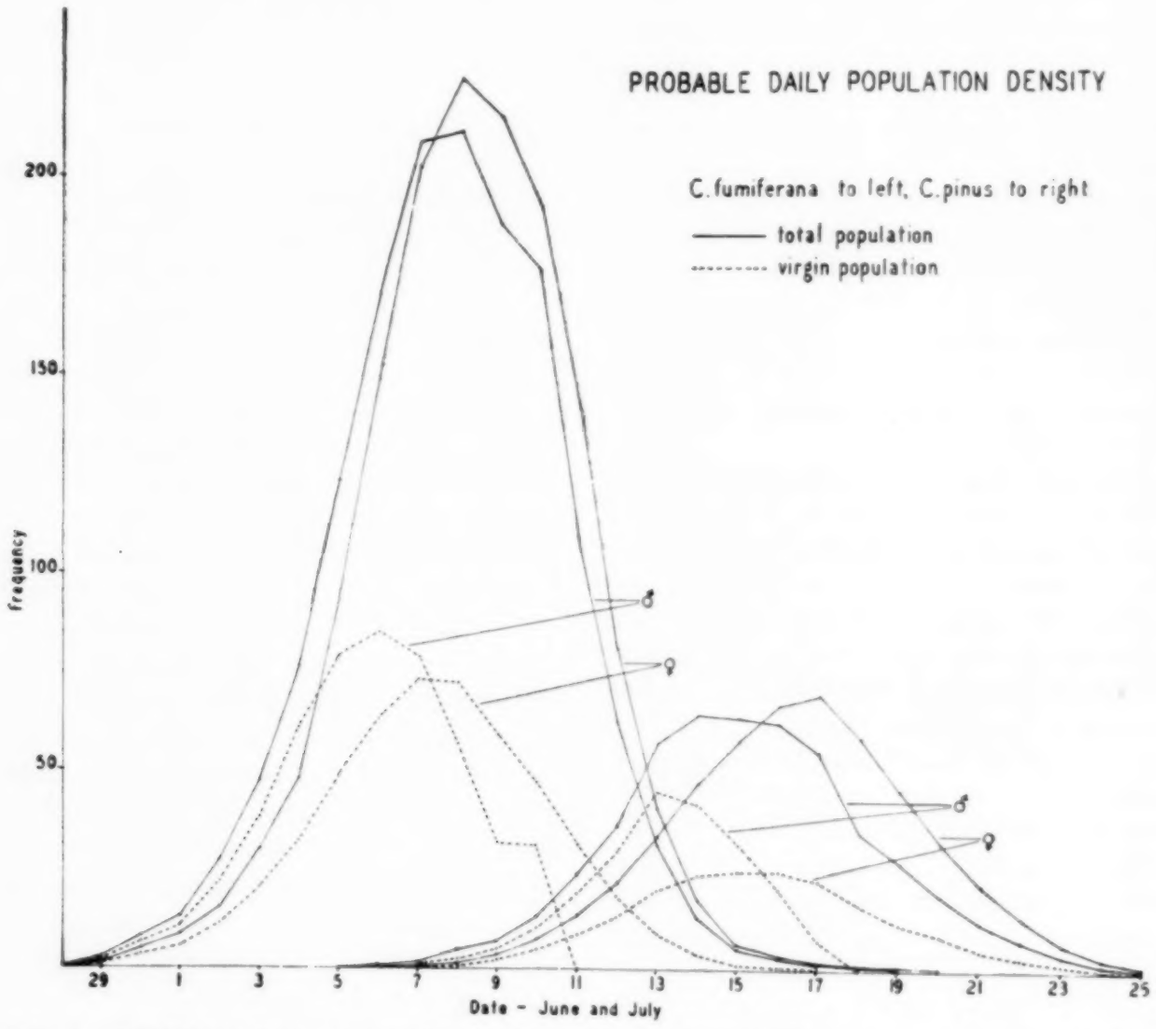

FIG. 3. Probable daily frequency distribution of *C. fumiferana* and *C. pinus* by sexes adjusted to an overall 3 : 1 basis (see text).

males and mated females. The 8 distributions, for mated and unmated males and females of the 2 species, are reproduced graphically in figure 3. It is appreciated that the true curves should probably be somewhat skewed, with the right-hand components trailing off, but, from the point of view of the proportions of the 2 populations that overlap, the assumption that the distributions are fairly normal about the means provides reasonable approximations for purposes of analysis. It should be mentioned that, because the observed numbers at opposite ends of the original distributions are unequal, no direct method of arriving at values for intercalation on intermediate days is available; the theoretical expectations over this area have therefore been taken as half the sum of the alternative values. This is, admittedly, somewhat arbitrary but from the forms of the derived curves it is quite clear that they provide more valid estimates of the true population densities than do the actual numbers obtained by the somewhat crude method of sampling.

As has been stated, the errors inherent in the sampling procedure provide a false impression of the relative frequencies of the 2 species. The data plotted in figure 3 and the overall populations in tables 5 and 6 have therefore been adjusted in the proportion of 3 *fumiferana* to 1 *pinus*. The validity of these proportions is attested to by the relative frequencies of mating pairs (see later), which latter, besides being more readily detectable than solitary individuals, are not subject to pressure of high numbers. It is upon these distributions that further calculations are based.

To return to the question of the emergence differential in the field, because no method is available for determining the nuptial status of males, mean eclosion dates for *fumiferana* and *pinus* can be calculated only for females. Based on

TABLE 5. *Daily probability values for cage emerged female* Choristoneura fumiferana *and derived values for field population*

Date	Cage % ♀♀	Field virgin ♀♀	Field total ♀♀	Field mated ♀♀	Field total ♂♂	Field virgin ♂♂
June 29	.25	.67	.99	.32	1.58	1.26
30	.76	2.04	3.02	.98	4.82	3.84
July 1	1.25	3.35	4.98	1.63	7.94	6.31
2	2.60	6.96	10.34	3.38	16.50	13.12
3	4.50	12.04	17.90	5.86	28.57	22.71
4	7.20	19.26	28.63	9.37	45.69	36.32
5	10.50	29.11	55.60	26.49	73.50	47.01
6	13.30	38.05	89.06	51.01	102.11	51.10
7	14.80	43.85	121.50	77.65	124.60	46.95
8	13.70	41.98	133.97	91.99	125.93	33.94
9	11.20	35.41	128.18	92.77	111.75	18.98
10	8.70	28.34	114.77	86.43	105.09	18.66
11	5.60	18.80	84.16	65.36	65.32	−.04
12	3.20	10.74	48.06	37.32	37.30	−.02
13	1.60	5.37	24.05	18.68	18.67	−.01
14	.70	2.35	10.51	8.16	8.16	
15	.25	.84	3.75	2.91	2.90	−.01
16	.15	.50	2.25	1.75	1.75	
17	.07	.23	1.05	.82	.81	−.01
18	.02	.07	.29	.22	.22	
19	.01	.04	.16	.12	.12	
20			.09	.09		−.09
23			.02	.02		−.02
Total	100.36	300.00	883.33	583.33	883.33	300.00

TABLE 6. *Daily probability values for cage emerged female* Choristoneura pinus *and derived values for field population*

Date	Cage % ♀♀	Field virgin ♀♀	Field total ♀♀	Field mated ♀♀	Field total ♂♂	Field virgin ♂♂
July 6	.09	.12	.21	.09	.35	.26
7	.17	.23	.38	.15	.66	.51
8	.42	.56	.91	.35	1.60	1.25
9	.92	1.22	2.00	.78	3.52	2.74
10	1.85	2.47	4.02	1.55	7.08	5.53
11	3.25	4.33	7.07	2.74	12.45	9.71
12	5.15	6.86	11.20	4.34	19.72	15.38
13	7.75	10.33	16.86	6.53	29.72	23.19
14	9.90	12.18	24.35	12.17	33.72	21.55
15	11.20	12.60	29.85	17.25	33.59	16.34
16	12.30	12.58	34.41	21.83	32.18	10.35
17	12.50	11.49	35.66	24.17	28.18	4.01
18	10.70	8.73	30.29	21.56	20.49	−1.07
19	8.55	6.08	23.36	17.28	13.64	−3.64
20	6.05	4.30	16.50	12.20	9.64	−2.56
21	3.95	2.81	10.77	7.96	6.29	−1.67
22	2.45	1.74	6.69	4.95	3.90	−1.05
23	1.22	.87	3.34	2.47	1.96	− .51
24	.58	.40	1.57	1.17	.91	− .26
25	.15	.10	.42	.32	.26	− .06
Total	99.15	100.00	259.86	159.86	259.86	100.00

the corrected daily frequencies of virgin females (tables 5 and 6), the mean times of eclosion for *fumiferana* and *pinus* females are calculated to be July 7, 2340 hrs and July 16, 0315 hrs respectively, giving a difference of 8 d. 3 h. 35 m. instead of approximately 11 days as determined from cage eclosion data (table 3).

The expected overlap of adults materialized over an 18-day period extending from July 6 until July 23. It remains to determine what proportions of the 2 species, by sexes, contributed to this overlap.

When calculated from the theoretical population density curves, the proportions of the 2 species that occurred together differ from those based on cage emergence figures. This is obviously due to the cumulative effect of daily emergence in the field: there, unmated individuals of the 2 species co-existed over only 14 days (July 6–19, table 4); in cages the period was again 14 days (July 4–17, table 2). This affords strong support for the contention that virginity in nature is a short-term status for females of both species (see next section). Altogether about 85% of the adults of the 2 species were contemporaneous, as compared with only about two-thirds of the cage populations that coincided in emergence. However, eliminating those that had mated (table 8) and adjusting for the intraspecific sex differential in eclosion leaves *ca.* 80% of the unmated moths overlapping in the field—a not unreasonable fit to the value of 71% derived from cage emergence data. By either method of calculation, then, three-quarters provides a rough estimate of the extent to which the temporal barrier broke down in the plot under observation.

Hybridization potential

1. *Evidence from cage emergence data:* It has been assumed that the pattern of emergence in cages parallels that occurring in the field and thereby constitutes an estimate of the relative proportions of the 2 species by sex that are being introduced daily into the accumulating field

populations. These together with the unmated residues from preceding days are the proportions of the species that are susceptible to hybridization on any particular day that both species are present. Laboratory observations have adequately demonstrated both the avidity with which the newly emerged males engage in mating activity and the receptivity of females at least by the evening of the day on which they eclose. It therefore appears reasonable to assume that each day's output of females is mated on the day of eclosion by an equal number of the more abundant males, thus setting a limit to the number of unmated males that can be carried over to the following days.

With the object of arriving at a theoretical standard for potential hybridization, the cage data in table 2 are more open to analysis if, first, the differentials in the sex ratios are eliminated. It then remains only to adjust the relative frequencies of the 2 species to conform more nearly with those in the field: for reasons that have been given, a rough estimate is taken to be 3 times as many *fumiferana* as *pinus* moths. Accordingly, for purposes of analysis the cage distribution of the former is based on 300 of each sex, while that of the latter is given in straight percentages.

It will be seen from table 2 that the overall emergence period may be subdivided into 5 groups of days depending on what combinations of sexes and species were present: (a) *fumiferana* males and females alone (June 28 to July 3); (b) *fumiferana* males and females with *pinus* males (July 4 and 5); (c) both sexes of both species (July 6 to 14); (d) *fumiferana* females with *pinus* males and females (July 15 to 17); and (e) *pinus* males and females alone. At the onset of mating only *fumiferana* matings are possible, and, since males are in excess, the unmated residue is carried over into period (b). On these days, the newly emerged and the residual *fumiferana* males are in competition with *pinus* males for the *fumiferana* females that have eclosed. In period (c) both complements of newly emerged females occur with males of both species (residuals and newly emerged), and during (d), although no fresh *fumiferana* males appear, the residue from period (c) competes with *pinus* males for new complements of females. In the final period, (e), *fumiferana* female emergence is over, but residual males are accommodated by the proportional excess of *pinus* females.

With mating entirely at random, that is, in the absence of sexual isolation, periods (b) to (e) produce types of intra- and inter-specific unions whose proportions are governed by the frequencies in which the species and sexes are present. For example, in period (c), potential *fumiferana* matings are: 88.5 *f.* ♀♀ × 100.0 *f.* ♂♂ + 48.9 *p.* ♂♂, or 59.4. The remaining 29.1 *fumiferana* females are taken by *pinus* males. Similarly the *pinus* females of period (c) copulate with *pinus* and *fumiferana* males to provide 8.4 *p.* × *p.* and 17.1 *p.* × *f.* matings. Since the 4 types of matings account for all the females but only 76.5 *f.* and 37.5 *p.* males, there remains a balance of each, 23.5 *f.* and 11.4 *p.*, to be carried forward into the next mating period. The analysis of all 5 periods is given in table 7, together with a summation for the entire cage emergence period.

Since 89.1 of the 262.8 conspecific *fumiferana* matings are consumated before *pinus* adults enter the population and since an excess of 23.8 *pinus* females remains for mating with *pinus* males during the final mating period, it is only the balance of the cage population, namely 574.2 or 71.8%, that is in fact open to hybridization owing to the breakdown of the temporal barrier. Moreover, by taking into account the numerical disproportion of the species and sexes from period to period, the hybridization potential is effectively reduced from about 36 (or 35.5, *v.* previous section) to 18.6%.

2. Evidence from field populations: The hybridization potential in the field can be estimated only on the basis of the relative

TABLE 7. *Hybridization potential determined from cage emergence data (weighted according to field densities)* *

Period		*C. f.* ♀♀	*C. f.* ♂♂	*C. p.* ♀♀	*C. p.* ♂♂	Potential mating frequency *f*♀×*f*♂	*f*♀×*p*♂	*p*♀×*f*♂	*p*♀×*p*♂
June 28–July 3	Emerged	89.1	161.4						
	Mated	89.1	89.1			89.1			
July 4 and 5	Residue	0.0	72.3						
	Emerged	112.5	81.3		2.2				
	Total	112.5	153.6		2.2				
	Mated	112.5	110.9		1.6	110.9	1.6		
July 6–14	Residue	0.0	42.7		0.6				
	Emerged	88.5	57.3	25.5	48.3				
	Total	88.5	100.0	25.5	48.9				
	Mated	88.5	76.5	25.5	37.5	59.4	29.1	17.1	8.4
July 15–17	Residue	0.0	23.5	0.0	11.4				
	Emerged	9.9	0.0	46.7	33.2				
	Total	9.9	23.5	46.7	44.6				
	Mated	9.9	19.5	46.7	37.1	3.4	6.5	16.1	30.6
July 18–25	Residue	0.0	4.0	0.0	7.5				
	Emerged		0.0	27.8	16.3				
	Total		4.0	27.8	23.8				
	Mated		4.0	27.8	23.8			4.0	23.8
Total	Residue		0.0	0.0	0.0	262.8	37.2	37.2	62.8
Per cent						65.7	9.3	9.3	15.7

* The assumed field densities are 3 *fumiferana* to 1 *pinus*.

daily frequencies of unmated moths: the total daily populations, being in part cumulative, include individuals that are already mated and thus provide overestimates. The calculated daily number of *fumiferana* virgin males and females, taken from table 5, and those for *pinus*, taken from table 6, are grouped in table 8. Analyzing these distributions in the same way as was done with the cage eclosion data, except that each day's contribution is calculated separately, establishes the hybridization potential for the population as a whole at 11.5%, or, during the period when virgins of opposite species co-exist, at 14.5%.

It has already been pointed out that the efficacy of temporal isolation is only about 20%, yet, despite some 80% of the overall virgin population being open to hybridization, instead of 40% hybrids, only 11.5% are theoretically possible: this is quite obviously due to the numerical disproportion of the species and sexes on different days.

Sexual Isolation

Sexual isolation is an obvious and important barrier to hybridization between any sympatric populations of sibling species. Its partial breakdown under experimental conditions has been observed for *fumiferana* and *pinus* (Smith, 1953) but no direct information as to its efficacy in nature has previously been available.

The psychological component: Combining the results obtained during both the population and the mating censuses, 285 budworm pairs were recorded between June 29 and July 22 inclusive. Records on 246 of these were compiled during the period from July 6 to 19, when unmated individuals of both species were contemporaneous. The 246 comprised 170 *fumiferana* pairs and 76 *pinus* pairs. No hybrid unions were found, so that, despite the expectation of 14.5%, or some 36 among the 246 observed, sexual isolation between the 2 species was in fact complete.

The differential in daily mating time: The distribution of the recorded matings by day and by hour is given in table 9. The moderate preponderance of *fumiferana* matings at 1500 hrs. on "long" days as compared with 1600 hrs. is attributable to the accumulation of matings that had been initiated earlier. The second set of figures at 1500 hrs. lists the numbers of matings recorded during the routine assessment of population density; here the component numbers are likewise higher because the trees were sampled only once, at about 1500 hrs., and then for 30 minutes.

The mean mating hours of the 2 species, calculated only from matings observed between July 6 and 22 (table 9) and omitting those recorded during population census taking, are 1651 and 1854 for *fumiferana* and *pinus* respectively. The 2-hour differential between the 2 species in nature thus parallels that obtaining under experimental conditions (Smith, 1953). It will be noted that the later time for *pinus* is reflected also in that as many as 10 of the 76 *pinus* pairs occurred after 2100 hrs., whereas only 2 out of the 170 *fumiferana* matings recorded during the period of overlapping flight occurred that late. Or again, 100 of the 170 *fumiferana* matings were underway by 1500 hrs., whereas only 17 of the 76 *pinus* were initiated at that time.

The observed numbers of *fumiferana* and *pinus* adults *in copula* unfortunately do not provide ready estimates of the relative frequencies of the 2 species. This is because long-day records were not

TABLE 8. *Hybridization potential determined from probability values for daily field virgin males and females*

Date	*C. fumiferana*		*C. pinus*		Potential mating frequency			
	♀ ♀	♂ ♂ +resid.	♀ ♀	♂ ♂ +resid.	*f.*×*f.*	*f.*×*p.*	*p.*×*f.*	*p.*×*p.*
June 29–								
July 5	73.43	130.57			73.43			
6	38.05	51.10+57.14	.12	.26	37.96	.09	.12	
7	43.85	46.95+70.16	.23	.51+ .17	43.60	.25	.23	
8	41.98	33.94+73.28	.56	1.25+ .43	41.33	.65	.55	.01
9	35.41	18.98+65.34	1.22	2.74+ 1.02	33.90	1.51	1.17	.05
10	28.34	18.66+49.25	2.47	5.53+ 2.20	25.44	2.90	2.22	.25
11	18.80	−.04+40.25	4.33	9.71+ 4.58	13.87	4.93	3.20	1.13
12	10.74	−.02+23.14	6.86	15.38+ 8.23	5.31	5.43	3.40	3.46
13	5.37	−.01+14.41	10.33	23.19+14.72	1.48	3.89	2.84	7.49
14	2.35	+10.08	12.18	21.55+26.53	.41	1.94	2.11	10.07
15	.84	−.01+ 7.56	12.60	16.34+36.07	.11	.73	1.59	11.01
16	.50	+ 5.85	12.58	10.35+40.67	.05	.45	1.30	11.28
17	.23	−.01+ 4.50	11.49	4.01+39.29	.02	.21	1.08	10.41
18	.07	+ 3.39	8.73	−1.07+32.68	.01	.06	.85	7.88
19	.04	+ 2.53	6.08	−3.64+23.67		.04	.68	5.40
20–25		−.11+ 1.85	10.22	−6.11+14.59			1.74	8.48
Total	300.00	300.00	100.00	100.00	276.92	23.08	23.08	76.92
Per cent					69.23	5.77	5.77	19.23

taken for the former species until *pinus* adults appeared. However, using the proportion of 1500 hr. matings against the total *fumiferana* pairs observed between July 6 and 18 inclusive, the unrecorded *fumiferana* matings between June 29 and July 5 can be calculated at about 30. Hence the overall mating frequencies that would probably have been recorded were long-day observation to have included the early part of the *fumiferana* season approximate 235 *fumiferana* pairs and 80 *pinus* pairs, or about 3 to 1. This is the ratio that has been taken as representing the actual frequencies of the 2 species in the field.

Indices of Reproductive Isolation

Isolation indices provide mathematical measurements of the efficacy of the various contributory factors that together tend to restrict or prevent gene exchange between sympatric populations of closely related species. In 1 type of experiment investigating sexual isolation, the value of the index is derived from the number and types of matings resulting from the confinement of members of both sexes of 1 species with those of a second. The number of intra- minus inter-specific matings divided by the total number then provides a measurement that may range from + 1.0, where isolation is complete, to 0, where no preferential mating results.

Here it is proposed to extend the procedure to an assessment of the ecological and temporal components encountered in this study. The only limitation to its use lies in the adequacy of the sampling techniques used or, in this particular instance, in the validity of the correction factors applied to compensate for the obvious deficiencies

TABLE 9. *Frequency of* fumiferana (*f*) *and* pinus (*p*) *pairs* in copula *at specified hourly intervals*

Date	1200		1300		1400		1500		1600		1700		1800		1900		2000		2100		2200		2300		2400	
	f	*p*	*f*	*p*	*f*	*p*	*f*	*p*	*f*	*p*	*f*	*p*	*f*	*p*	*f*	*p*	*f*	*p*	*f*	*p*	*f*	*p*	*f*	*p*	*f*	*p*
June																										
29							0+1																			
30							0+3																			
July																										
1							0+2																			
2							0+4																			
3							0+2																			
4							0+8																			
5	2		2		2		3+6																			
6	3	0	0	0	2	0	4+11	0+0	(Rain)				1	0	1	0	2	0	3	0	0	0				
7							6+9	0+0	4	0	5	0	2	0	3	0	2	0	1	0	1	0	0	0		
8	1	0	1	0	3	0	3+14	0+0																		
9							3+5	0+0	5	0	4	0	5	0	2	0	1	0	1	0	1	1	0	0		
10	0	0	0	0	3	0	2+4	0+0																		
11							3+3	0+0	2	0	2	1	0	2	1	1	0	3	1	2	0	0	0	0		
12	0	0	0	0	(Rain)		0+1	0+0																		
13							4+4	1+1	3	2	0	4	0	0	5	3	3	2	2	4	0	1	0	1	0	0
14	0	0	1	0	0	0	1+2	2+2																		
15							2+2	0+1	1	1	0	1	1	2	2	4	1	2	1	3	0	4	0	2	0	1
16	0	0	0	0	0	0	1+0	0+0																		
17					0	1	0+0	1+1	0	1	0	2	1	0	0	2	0	4	0	1	0	0				
18	0	0	0	0	0	0	1+1	2+2																		
19							0+0	1+2	0	0	0	0	0	2	0	0										
20	0	0	0	0	0	0	0+0	0+0																		
21							0+0	0+0	0	0	0	0	0	0	0	2	0	0								
22	0	0	0	0	0	0	0+0	1+0	0	0	0	1	0	0	0	0										
23	0	0	0	0	0	0	0+0	0+0	0	0	0	0	0	0	0	0										
24	0	0	0	0	0	0	0+0	0+0																		
	6	0	4	0	10	1	33+82	8+9	15	4	11	9	10	6	14	12	9	11	9	10	2	6	0	3	0	1

TABLE 10. *Indices of adult ecological isolation*

Species	Sex	Number on Bf	Number on Pj	Indices	Joint indices (3 : 1 basis)	
fumiferana	♂	493	32	+ .878		
pinus	♂	7	193	+ .930	+.891	
fumiferana	♀	283	2	+ .986		+.940
pinus	♀	0	134	+1.000	+.990	

in the sampling methods employed. Since the corrections, both within and between populations, are admittedly only approximate, the measurements themselves will be correspondingly inexact but, as is by now evident, this is of little consequence because the sexual barrier to hybridization proved to be absolute.

Indices of ecological isolation

1. *Larval:* The mass collections taken off Bf and Pj provided a total of 510 adults, 292 from Bf and 218 from Pj, of which 7 *fumiferana* occurred on Pj. Based on relative population densities of 3 to 1, the index of larval ecological isolation is (654 + 211) − 7 over 872, or + .984, and is attributable to intrinsically different food preferences (*v.* Smith, 1953).

2. *Adult:* If adult virgin females were entirely confined to their preferred host trees, yet males were both completely free of this encumbrance and practiced no sexual selection, the index of adult ecological isolation would be precisely + .500. Omitting spent females and probable accidental transpositions, there were 41 individuals out of a total of 1144 that were recorded as being indiscriminant to tree species. In table 10 these totals, broken down by species and sex and also following conversion to a basis of 3 *fumiferana* to 1 *pinus*, have appended to them the calculated ecological isolation indices.

The index of temporal isolation

Theoretical values for this index range from + 1.0, where unmated moths of different species fail to occur together, through 0, where exactly half of each species coincide in eclosion, to − 1.0, where the eclosion periods are precisely co-ordinated. Under experimental conditions, both sexes of *fumiferana* have, with obvious reluctance on the part of already mated females, been compelled to engage in polygamy: a number of logical reasons force one to regard this as an abnormal situation, so that its common occurrence in nature may reasonably be discounted. Consequently in determining the value of temporal isolation, one is concerned only with virgin moths.

The weighted cage emergence data in table 7 set a value of − .436 to the index of temporal isolation. That for the adjusted field distributions is a comparable − .590.

The index of sexual isolation

Since no hybrid matings occurred among the 246 observed during the period when virgin individuals of both species were present, it is obvious that sexual isolation between *fumiferana* and *pinus* is absolute under field conditions and the index accordingly stands at + 1.0. This value is to be compared with the potential value of + .710 calculated for a panmictic population in which the temporal barrier was only 20% effective (table 8).

DISCUSSION AND CONCLUSIONS

Dobzhansky (1951, p. 179) propounded the maxim "Race and species formation without isolation is impossible" and the corollary that the maintenance of species and races as distinct populations is contingent on their continued isolation. Patterson and Stone (1952, p. 545) express themselves thus: "Isolating mechanisms are the *sine qua non* for evolution of genetically separate strains of sympatric

organisms." It is, of course, with Dobzhansky's corollary alone that we are immediately concerned.

That the spruce budworm and the jack-pine budworm have diverged to such a degree that they now constitute discrete biological entities has been conceded for a number of years (Graham, 1935; Smith, 1949) and has finally received formal taxonomic recognition, (Freeman, 1953). The role played by geographic isolation being self-evident, recent studies of the 2 species have been concerned predominantly with determining what intrinsic, genetically conditioned, properties are responsible for maintaining their integrity (Smith, 1953). With the discovery of a potential breakdown of temporal isolation at Blue and Indian Lakes in the spring of 1952, this question assumed a status of paramount importance, for the onus would thereby be shifted more squarely on to the ecological and sexual components of reproductive isolation.

In contemplating on the possible consequences of failure of the seasonal differential, Smith (1953) pointed out that the absence of obvious hybrids from among natural populations strongly implies either that the temporal barrier is inviolable or that sexual isolation and/or the different ecological requirements of the 2 species must under such circumstances assume an all-important function in preventing gene exchange between them. It was stressed that laboratory determinations are of dubious predictive value regarding the probable effectiveness of sexual isolation in the field, and it was suspected that difficulty might be experienced in any attempt to weigh the relative merits of the sexual and ecological factors even under natural conditions. Such perhaps has not proved to be the case.

As was stated in the Introduction, the partial breakdown in temporal isolation is attributed to the abnormally high temperatures that prevailed before and at the time of larval emergence from diapause. Limited evidence from experimental stock material points to *pinus* having the higher temperature threshold for emergence from diapause, and the differential between the 2 species seems quite definitely inversely related to temperature. The lowering of the temporal barrier is thus attributable to an initial failure of phenological isolation; judging by the 11-day differential in mean cage-emergence time, the phenological breakdown was in some degree compensated for by subsequent ontogenetic isolation.

Laboratory observation, making use of "mate-choice" techniques, has fully demonstrated that the innate repugnance to cross-mating is expressed solely by females of both species, and that it is reinforced by their reluctance to deviate from an ingrained species-specific differential in mating time, a reluctance that is in no way shared by males. That the latter partial isolation factor is equally operative in the field has already been established herein. That the males are essentially devoid of discrimination was particularly well exemplified by 2 bizarre sets of sexual incongruities. One involved attempts by males to engage in copulation with dead females of either species. In the second category were infrequently observed but invariably unsuccessful attempts by males of both species to participate in the mating activity of already associated pairs of *Malacosoma disstria* Hbn., which were common at the time. Since no approaches were made to solitary or dead *disstria* females, it is difficult to escape the conclusion that the sexual act under way was the motivating force behind the promiscuous advances. The fact of the dead *Choristoneura* females that incited males of the opposite species being usually on their appropriate host trees casts some doubt on ecological isolation as a major factor in preventing hybridization. Also, apart from the fortuitous transposition of a *pinus* mating pair from Pj to Bf, the observation of a single conspecific *fumiferana* mating taking place on a Pj tree strongly suggests that ecological isolation is a factor in species preservation only in so far as it is a usual

concomitant of geographical isolation [2] (on this point see Mayr, 1947). Allee *et al.* (1949, p. 610) have stressed the necessity for careful study and analysis of sympatric species that occupy different habitats, because genetic isolation may be primary and ecological isolation secondary: this is clearly so in the present case.

It was earlier contended (Smith, 1953) that, owing to the sex differential in time of maturing, were temporal isolation and, then, sexual isolation to lapse in nature, it is the *fumiferana* ♀ × *pinus* ♂ hybrid that has the better chance of materializing. These current observations afford some support for this contention, although the extent of temporal breakdown and the method of analysis adopted to determine the hybridization potential, neglecting as the latter does the question of longevity, together tend to obscure the point.

The previous review of isolating mechanisms concluded with the following statement: "That hybridization does not occur in nature is made virtually certain from our knowledge of the efficacy of reproductive isolation: whether in fact it does not is for the future to decide and for population genetics to determine." The results of this field study might well be summarized: sexual isolation is the component of reproductive isolation that renders these 2 species of *Choristoneura* mutually exclusive genetic systems.

[2] In a review of my earlier paper, Remington (1953) has speculated on the way in which these species evolved. It seems unwarranted to place emphasis on the "chromosomal differences" and on "nutritional inviability of hybrids" as causal agents in population separation as species. Rather than entertain the idea of these as "the most important factors," the following sequence might be more consistent with what is known: initial ecological and almost contemporaneous geographical isolation (as populations infiltrated essentially pure fir and pure pine areas) leading to temporal isolation (as the populations because selectively adapted to the different phenologies of the 2 hosts); during this time, changes in gene balance and sequence presumably arose, which only following the restoration of sympatry conferred sufficiently deleterious effects on hybridization for the development of sexual isolation to become obligatory.

Summary

The closely related species *Choristoneura fumiferana* (on balsam fir) and *C. pinus* (on jack pine) will hybridize readily in captivity but show no evidence of introgression having occurred in Nature. The integrity of sympatric populations has therefore been attributed to ecological, temporal, and sexual isolation. In 1952, a localized breakdown of the temporal barrier afforded an opportunity to assess the contributions to complete reproductive isolation made by the 3 components.

Indices of isolation were as follows: larval ecological + .984, adult ecological + .940; temporal isolation of adults − .590, which provided a hybridization potential of 11.5% for the field population as a whole, or 14.5% for contemporaneous virgins. Despite this, only conspecific unions materialized.

Sexual isolation is therefore the component of reproductive isolation that renders the 2 species mutually exclusive genetic systems.

Acknowledgment

It is indeed a pleasure to acknowledge my indebtedness to Forest Biology Ranger W. J. Miller: he was in large measure responsible for the early discovery of the overlap-potential, and, despite a heavy work-schedule, willingly undertook the extra collecting that established with virtual certainty that a temporal breakdown would materialize.

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EXPERIMENTAL STUDIES OF THE DISTRIBUTION OF GENE FREQUENCIES IN VERY SMALL POPULATIONS OF *DROSOPHILA MELANOGASTER*. II. BAR[1]

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Received February 23, 1954

History by generations

The results from the experiments with Bar, to be described here, present a more complicated situation than those from similar experiments with forked, described in part I of this series (Kerr and Wright, 1954) because of strong selective pressures of various sorts. A more complete analysis can be made, however, because of the possibility of distinguishing accurately all genotypes.

108 lines, each starting from 4 B/+ females and 2 B/0, 2 +/0 males, were carried either to fixation or to generation 10 by random selection of 4 females and 4 males as parents in each generation. The methods were as described in part I. In 3 cases, flies selected as parents included ones that should not have appeared under simple sex linked heredity. These could have been due to nondisjunction but also possibly to nonvirginity of the females. These 3 vials are omitted in the analysis of selection coefficients but as no important changes in gene frequency were involved, the later generations from them have been included.

Table 1 shows the distribution of frequencies of the Bar gene by generation without omission of any vials. It may be seen that wide variability in F_1 was followed by fixation of type in 15 vials and of Bar in one vial in F_2. In generation 10, type had become fixed in 95 lines and Bar in three, while only 10 lines remained unfixed. It is evident that selection strongly favors type but that Bar can occasionally drift into fixation in spite of this.

A χ^2 test of the distributions of unfixed and newly fixed classes by generation from generation 4 to 10 (grouping generations 7 and 8, 9 and 10, classes 5 and 6, 7 to 12) yields a value of 22.2, 24 degrees of freedom and a probability between .50 and .70. It is evident that equilibrium of the form of the distribution was substantially reached by generation 4 and that an average of 22.0% of the unfixed classes became fixed per generation with respect to type and 0.7% with respect to Bar.

The selection coefficients

Table 2 shows the results from vials according to the arrays of parents. The experiment was not designed for analysis of the mode of selection but something can be deduced. The small numbers of flies counted per vial makes it necessary to consider many vials to obtain significant results but has the advantage that the results do not depend much on the peculiarities of selection in particular cases. F_1 (mothers 040, fathers 22) is here excluded as it is desirable to separate it from the others because of possible systematic differences.

Tables 3 and 4 show the numbers of Bar and type sons produced by different groups of females (no B/B in (3), B/B

[1] Experimental data by W. E. Kerr under a fellowship of the Rockefeller Foundation; mathematical analysis by S. Wright. Acknowledgment is made to Dr. Th. Dobzhansky for material and for his hospitality during the conduct of these experiments. Acknowledgment is also made to Dr. J. Crow for material and suggestions. Analysis was aided by a grant from the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

TABLE 1. *The distribution of numbers of Bar genes represented in each generation among 108 lines consisting of 4 males, 4 females each.* Newly fixed genes are distinguished from those fixed in previous generations.

No. Bar genes		Generation 0	1	2	3	4	5	6	7	8	9	10	4–10	%
Old	0				15	30	43	55	65	78	88	91		
New	0			15	15	13	12	10	13	10	3	4	65	22.0
	1		8	12	10	17	10	11	7	5	3	4	57	19.3
	2		7	13	16	14	14	6	3	1	2	2	42	14.2
	3		24	22	10	5	9	8	6	2	4	1	35	11.9
	4		23	20	16	10	6	4	3	2	1	1	27	9.2
	5		21	11	13	4	2	3	4	4	2	1	20	6.8
	6	108	17	8	5	7	4	5	2	1	—	—	19	6.4
	7		3	3	3	3	3	2	2	1	1	1	13	4.4
	8		3	2	3	2	—	2	1	—	—	—	5	1.7
	9		2	—	1	2	3			1	1	—	7	2.4
	10			1			1			—	—	—	1	0.3
	11			—						1	1	—	2	0.7
New	12			1				1		—	—	1	2	0.7
Old	12				1	1	1	1	2	2	2	2		
Total		108	108	108	108	108	108	108	108	108	108	108	295	100.0

TABLE 2. *The offspring of 4 males, 4 females picked at random, from vials of each type.* The column 004 refers to vials in which there were 0 B/B, 0 B/+ and 4 +/+ females and similarly with the others. The row 04 refers to vials in which there were 0 B/0 males, 4 +/0 males, and similarly with the others.

Fathers	Offspring	Mothers 004	013	022	031	040	103	112	121	130	211	220	301	310	400
04	B/B		0	0	0	0	0	0	0	0		0			
	B/+		31	26	13	28	0	1	7	4		1			
	+/+		181	78	23	20	4	11	5	4		3			
	B/0		17	28	11	19	0	4	4	3		2			
	+/0		195	76	25	29	4	8	8	5		2			
13	B/B	0	1	3	2	1	0	0	0	2	4	0	3		
	B/+	11	20	35	34	14	6	7	7	8	11	6	1		
	+/+	113	111	90	44	13	10	17	13	6	5	6	0		
	B/0	0	27	39	27	15	2	7	10	8	11	7	4		
	+/0	124	105	89	53	13	14	17	10	8	9	5	0		
22	B/B	0	6	5	7	6	0	3	5	1	0	5	0	2	
	B/+	26	35	58	34	31	0	7	7	12	2	12	8	9	
	+/+	54	111	69	59	31	8	6	8	3	2	3	0	1	
	B/0	0	12	43	40	31	0	4	7	7	0	13	3	6	
	+/0	80	140	89	60	37	8	12	13	9	4	7	5	6	
31	B/B	0	0	7	7	6		3	7	1		6		4	1
	B/+	4	15	32	29	9		4	17	5		7		4	3
	+/+	8	17	25	20	5		1	4	2		3		0	0
	B/0	0	0	18	27	13		4	15	5		12		8	4
	+/0	12	32	46	29	7		4	13	3		4		0	0
40	B/B		1	0		4			1	4				4	
	B/+		15	4		4			3	8				0	
	+/+		0	0		0			0	0				0	
	B/0		3	3		4			2	6				4	
	+/0		13	1		4			2	6				0	

TABLE 3. *Sons from vials with no B/B mothers.* Viability (V_{B0}) of B/0, relative to +/0, estimated on the hypothesis of no differential productivity. Productivity (U_{B+}) of B/+, relative to +/+, estimated on the hypothesis of no differential viability. Expectation on the hypotheses $V_{B0} = 1$, $U_{B+} = 1$.

Mothers			Sons							Sons (exp.)	
B/B	B/+	+/+	B/0	+/0	Total	V_{B0}	SE ($V_{B0}=1$)	U_{B+}	SE ($U_{B+}=1$)	B/0	+/0
0	1	3	59	485	544	.85	.11	.83	.15	68.0	476.0
0	2	2	131	301	432	1.31	.13	1.54	.17	108.0	324.0
0	3	1	105	167	272	1.05	.15	1.13	.31	102.0	170.0
0	4	0	82	90	172	.91	.13	—	—	86.0	86.0
Total (excl. 0	4	0)	295	953	1248			1.094	.105	278.0	970.0
Total (incl. 0	4	0)	377	1043	1420	1.053	.064			364.0	1056.0

TABLE 4. *Sons from vials containing B/B mothers.* Productivity (U_{BB}) of latter estimated on hypothesis that $U_{B+} = 1$, $V_{B0} = 1$. Expectation on these hypotheses and the hypothesis that $U_{BB} = .37$.

	Mothers			Sons						Sons (exp.)	
	B/B	B/+	+/+	B/0	+/0				SE	B/0	+/0
Classes						Total	U_{BB}	SE	($U_{BB}=.37$)		
4	1	–	–	88	144	232	.41	.15	.14	86.0	146.0
2	2	–	–	45	31	76	.41	.19	.18	43.9	32.1
2	3	–	–	25	11	36	.41	.18	.17	24.6	11.4
Total 8				158	186	344	.408	.098	.092	154.5	189.5

present in (4)). Tables 5 to 9 show the numbers of B/B, B/+ and +/+ daughters from various groups of parents (no B/B mothers or B/0 fathers in (5), no B/B mothers or +/0 fathers in (6), at least one B/B mother present, fathers all +/0 or all B/0 in (7), no B/B mothers, mixed fathers in (8) and at least one B/B mother, mixed fathers in (9)). Table 2 must be referred to for a detailed account of the types of mating in these cases.

These are analyzed in terms of the following parameters:

In cases in which all three types of daughters are expected the analysis has been made in terms of genes: $p_B = p_{BB} + (1/2)p_{B+}$, $p_+ = p_{++} + (1/2)p_{B+}$, $n_B = 2n_{BB} + n_{B+}$ and $n_+ = 2n_{++} + n_{B+}$.

The method used has been to find the value of the parameter under consideration which maximizes the probability of the observed numbers. It has been shown that this method does not always give the best estimates in such an absolute sense as supposed by Edgeworth (1908) and Fisher (1921) (method of

	B/B	B/+	+/+	B/0	+/0
number of parents in vial	f_{BB}	f_{B+}	f_{++}	f_{B0}	f_{+0}
relative productivity of parents	U_{BB}	U_{B+}	1	U_{B0}	1
relative viability of offspring	V_{BB}	V_{B+}	1	V_{B0}	1
probability of offspring genotype	p_{BB}	p_{B+}	p_{++}	p_{B0}	p_{+0}
observed number of offspring	n_{BB}	n_{B+}	n_{++}	n_{B0}	n_{+0}

n_T is the sum of the n's in any case.

"maximum likelihood") (cf. Neyman, 1951) but in such a case as the present there seems to be no better method, with the qualification that we have not attempted any simultaneous determinations.

As the results from any one population type are here reduced to two alternatives, the quantity to be maximized is of the form $p_1^{n_1}p_2^{n_2}$. The equation to be solved for a productivity coefficient U (and similarly for a viability coefficient V) is thus

$$n_1 \frac{d}{dU} \log p_1 + n_2 \frac{d}{dU} \log p_2 = 0$$

The best estimate from a series of population types is obtained by iteration on equating the sum of such expressions to 0.

The variance from a single population type is given by the formula

$$\frac{1}{\sigma^2} = -(n_1 + n_2)\left[p_1 \frac{d^2}{dU^2} \log p_1 + p_2 \frac{d^2}{dU^2} \log p_2\right]$$

on substituting 1 for U (or V), if it is concluded that the difference from 1 is negligible, or by substituting the value of U (or V) that has been obtained, if this seems to differ significantly from 1. The variance for the estimate from a series of population types is obtained from the sum of such terms.

As the individual fly is taken as the unit, no cognizance is taken of possible heterogeneity among vials. The standard errors would be larger if such heterogeneity exists. The factor cannot be large in view of the uniform small number (4) of flies of each sex counted in each vial.

From the fourth entry in table 3, it may be seen that vials in which all 4 females were B/+ produced 82 B/0; 90 +/0 sons indicating a relative viability of .91 for B/0 which does not differ significantly from 1. In F_1, however, there were 192 B/0 : 240 +/0 sons from B/+ females, which differs from equality by 2.3 times its standard error. It indicates a relative viability of .80 for B/0. The results from the other entries in this table can be interpreted either on the basis of differential viability of the B/+ and +/0 sons or of differential productivity of the B/+ and +/+ mothers or a combination.

In estimating as differential viability it is convenient to use $r = f_{B+}/(f_{B+} + 2f_{++})$.

$$\left(\frac{rV_{B0}}{1 + rV_{B0}}\right)^{n_{B0}}\left(\frac{1}{1 + rV_{B0}}\right)^{n_{+0}}$$ is to be maximized.

$$\Sigma\left(\frac{1}{1 + rV_{B0}}\right)\left(\frac{n_{B0}}{V_{B0}} - n_{+0}r\right) = 0$$

$$\frac{1}{\sigma^2} = \Sigma[n_T r/V_{B0}(1 + rV_{B0})^2]$$

In estimating as differential productivity

$$\left[\frac{f_{B+}U_{B+}}{2(f_{B+}U_{B+} + f_{++})}\right]^{n_{B+}}\left[\frac{f_{B+}U_{B+} + 2f_{++}}{2(f_{B+}U_{B+} + f_{++})}\right]^{n_{+0}}$$

is to be maximized.

$$\Sigma\left[\left(\frac{f_{++}}{f_{B+}U_{B+} + f_{++}}\right)\left(\frac{n_{B0}}{U_{B+}} - \frac{n_{+0}f_{B+}}{(f_{B+}U_{B+} + 2f_{++})}\right)\right] = 0$$

$$\frac{1}{\sigma^2} = \Sigma\left[\frac{n_T f_{B+} f_{++}^2}{(f_{B+}U_{B+} + f_{++})^2(f_{B+}U_{B+} + 2f_{++})U_{B+}}\right]$$

The second entry in table 3 seems to indicate significantly superior viability of B/0 males or superior productivity of B/+ mothers but the total for all entries does not differ significantly from 1 on either basis. Other experimenters have

found inferior viability of Bar (e.g. Petit, 1951, $V_{B0} = .68 \pm .12$, Teissier $V_{B0} = .67$ as quoted by Petit). It is possible that there may be inferior viability of B/0 but superior productivity of B/+ in our data but the adoption of even a slightly lower viability B/0 leads to highly inconsistent and on the average very high estimates of productivity of B/+ though somewhat subnormal viability of the same (e.g. if $V_{B0} = .80$, U_{B+} comes out 1.52 and V_{B+} comes out .90). It is probable that there is some sort of selection advantage of the heterozygotes as indicated by the persistence of Bar for very long periods at very low frequencies in the experiments of L'Heritier and Teissier (1937). Petit (1951) and Teissier obtained selective values of $1.062 \pm .12$ and 1.24 respectively. We will however adopt the simplest hypothesis that is compatible with the present data, viz. that there is no differential viability of B/0 and +/0 males and no differential productivity of B/+ and +/+ females. The coefficients may of course be expected to have different values under different conditions.

The data of table 4 are interpreted on the above basis and thus as involving only possible differential productivity of B/B females relative to B/+ and +/+ collectively. The expression to be maximized in each case is

$$\left[\frac{2f_{BB}U_{BB} + f_{B+}}{2(f_{BB}U_{BB} + f_{B+} + f_{++})}\right]^{n_{B0}}\left[\frac{f_{B+} + 2f_{++}}{2(f_{BB}U_{BB} + f_{B+} + f_{++})}\right]^{n_{+0}}$$

$$\Sigma\left(\frac{f_{BB}}{f_{BB}U_{BB} + f_{B+} + f_{++}}\right)\left(\frac{n_{B0}(f_{B+} + 2f_{++})}{(2f_{BB}U_{BB} + f_{B+})} - n_{+0}\right) = 0$$

$$\frac{1}{\sigma^2} = \Sigma n_T\left[\frac{(f_{B+} + 2f_{++})f_{BB}^2}{(f_{BB}U_{BB} + f_{B+} + f_{++})^2(2f_{BB}U_{BB} + f_{++})}\right]$$

There was significantly low productivity of B/B ($U_{BB} = .41 \pm .10$). The number of Bar females seems to make no difference in the estimate.

We turn now to the deductions from the daughters. In table 5 with no B/B mothers, the results can be interpreted either as due to differential viability of B/+ and +/+ daughters or (again) as differential productivity of B/+ and +/+ mothers or a combination. The formulae are the same as those for the sons with obvious substitutions.

The results are much like those for the sons in table 3. The present data do not warrant any but the simplest hypothesis, viz. that there is no significant difference between B/+ and +/+ either in viability or productivity.

Table 6 shows the results for 28 females from vials in which all 4 fathers were B/0 and no B/B mothers were present. The results suggest low viability of B/B females ($.61 \pm .32$) in harmony with Petit and Teissier ($.75 \pm .15$, .84 respectively) but are obviously highly inadequate. It will be assumed for simplicity that there is no differential viability in view of the apparent absence of significant viability differences among the males in our own data.

Table 7 summarizes the few cases in which at least one B/B mother was present and the males were of a single genotype. These indicate very low productivity of B/B females. On combining with the earlier estimate, we arrive at $.369 \pm .087$ as the final estimate for U_{BB}.

Table 8 summarizes the data on daughters from vials in which B/B females were absent but both B/0 and +/0 males were present. It is assumed that there are no selective differentials except in productivity of B/0 and +/0 males. The frequency of the Bar gene among the daughters is theoretically the average of that in the gametes produced by the

TABLE 5. *Daughters from vials without B/B mothers and only +/0 fathers.* Interpreted alternatively on the hypotheses of no differential productivity of B/+ and +/+, or no differential viability of B/+ and +/+. Expectation on hypotheses: $V_{B+} = 1$, $U_{B+} = 1$.

Mothers			Fathers		Daughters								Daughters (exp.)	
B/B	B/+	+/+	B/0	+/0	B/B	B/+	+/+	Total	V_{B+}	SE ($V_{B+}=1$)	U_{B+}	SE ($U_{B+}=1$)	B/+	+/+
0	1	3	0	4	0	31	181	212	1.20	.24	1.24	.24	26.5	185.5
0	2	2	0	4	0	26	78	104	1.00	.23	1.00	.34	26.0	78.0
0	3	1	0	4	0	13	23	36	.94	.34	.87	—	13.5	22.5
0	4	0	0	4	0	28	20	48	1.40	.29			24.0	24.0
Total (excl. 0 4 0)					0	70	282	352			1.14	.19	66.0	286.0
Total (incl. 0 4 0)					0	98	302	400	1.131	.126			90.0	310.0

TABLE 6. *Daughters from vials without B/B mothers and only B/0 fathers.* Interpreted on hypothesis of no differential productivity of B/+ and +/+. Expectation on hypothesis: $V_{BB} = 1$, $U_{B+} = 1$.

	Mothers			Fathers		Daughters								
	B/B	B/+	+/+	B/0	+/0	B/B	B/+	+/+	Total	V_{BB}	SE	SE $V_{BB}=1$	Daughters (exp.) $V_{BB}=1$	
Classes														
3	0	–	–	4	0	5	23	0	28	.61 ± .32		.47	7.0	21.0	0.0

parents.

$$p_B = \frac{1}{2}\left[\left(\frac{f_{B0}U_{B0}}{f_{B0}U_{B0} + f_{+0}}\right) + \left(\frac{f_{B+}}{2(f_{B+} + f_{++})}\right)\right]$$

The expression to be maximized is $p_B{}^{n_B}(1 - p_B)^{n_+}$.

Let $A = f_{B0}f_{+0}/[2(f_{B0}U_{B0} + f_{+0})^2]$.

$$\Sigma A[2n_{BB} + n_{B+} - 2n_T p_B]/p_B(1 - p_B) = 0$$

$$\frac{1}{\sigma^2} = \Sigma[n_T A^2/p_B(1 - p_B)]$$

The data are classified both according to the relative numbers of B/+ and +/+ mothers and to the relative number of B/0 and +/0 fathers. In neither case is there a consistent trend. The grand average $U_{B0} = .264 \pm .040$ indicates very low productivity of B/0 males under these conditions. F_1 from 4 B/+ females; 2 B/0, 2 +/0 males in each vial, gave 28 B/B; 210 B/+ : 194 +/+ daughters, indicating even lower relative productivity of B/0 males (.125) than in the later generations.

The remaining data (table 9) are those

TABLE 7. *Daughters from vials including B/B mothers and either +/0 or B/0 fathers exclusively.* Interpretation on hypothesis of no differential viability. Expectation on this hypothesis and hypothesis that $U_{BB} = .37$.

	Mothers			Fathers		Daughters							Daughters (exp.)		
	B/B	B/+	+/+	B/0	+/0	B/B	B/+	+/+	Total	U_{BB}	SE	SE ($U_{BB}=.37$)	B/B	B/+	+/+
Classes															
5	1, 2	–	–	0	4	0	13	27	40				0	15.4	24.6
3	1, 3	–	–	4	0	9	11	0	20				11.3	8.7	0
8	1, 3								60	.126	.168	.276			
8	1, 3					(sons, table 4)			344	.408	.098	.092			
16	1–3								404	.369	.087	.087			

TABLE 8. *Daughters from vials without B/B mothers, classified according to numbers of B/+ and +/+ mothers or according to number of B/0 and +/0 fathers.* Interpretation on the hypothesis of no differential viability. Expectation on this basis and hypothesis that $U_{B0} = .26$.

	Mothers			Fathers		Daughters						Daughters (exp.)		
	B/B	B/+	+/+	B/0	+/0	B/B	B/+	+/+	Total	U_{B0}	SE	B/B	B/+	+/+
3	0	0	4	+	+	0	41	175	216	.36	.08	0	31.7	184.3
3	0	1	3	+	+	7	70	239	316	.18	.08	7.0	81.4	227.6
3	0	2	2	+	+	15	125	184	324	.31	.09	16.4	113.7	193.9
3	0	3	1	+	+	16	97	123	236	.15	.10	19.3	101.4	115.3
3	0	4	0	+	+	13	54	49	116	.23	.14	12.5	58.0	45.5
5	0	–	–	1	3	7	114	371	492	.25	.09	7.4	117.0	367.6
5	0	–	–	2	2	24	184	324	532	.28	.05	25.5	182.3	324.2
5	0	–	–	3	1	20	89	75	184	.24	.08	22.3	86.9	74.7
15	0	–	–	+	+	51	387	770	1208	.264	.040	55.2	386.2	766.6

in which B/B females and both type of males are present. The value $U_{BB} = .37$ is adopted from table 7 and the data used for an estimate of U_{B0} in the presence of at least one B/B female. The formulae are the same as above except that

$$p_B = \frac{1}{2}\left[\left(\frac{f_{B0}U_{B0}}{f_{B0}U_{B0} + f_{+0}}\right) + \left(\frac{2f_{BB}U_{BB} + f_{B+}}{2(f_{BB}U_{BB} + f_{B+} + f_{++})}\right)\right]$$

The results indicate significantly improved productivity of B/0 males when B/B females are present ($U_{B0} = .59 \pm .16$). Subdivision, according to the relative numbers of B/0 and +/0 males, suggest decline of productivity per B/0 male as the numbers of such males increase and subdivision according to the number of B/B females suggests increase of productivity per B/0 male as the number of B/B females increases, both in the direction of assortative mating, but not significantly.

Summing up, this analysis indicates that the present data can be interpreted adequately without assuming any differential viability, or any difference in productivity of B/+ and +/+ females but productivity of B/B females only 37% of that of B/+ or +/+, and productivity of B/0 males only 26% of that of

TABLE 9. *Daughters from vials including B/B mothers classified according to numbers of B/0 and +/0 fathers or numbers of B/B mothers.* Interpretation on hypothesis of no differential viability. Expectation on this basis and hypothesis that U_{B0} in presence of B/B females is .59.

	Mothers			Fathers		Daughters						Daughters (exp.)		
	B/B	B/+	+/+	B/0	+/0	B/B	B/+	+/+	Total	U_{B0}	SE	B/B	B/+	+/+
7	1–3	–	–	1	3	9	46	57	112	.66	.35	7.2	47.8	57.0
8	1–3	–	–	2	2	16	57	31	104	.58	.24	18.5	51.5	34.0
6	1–4	–	–	3	1	22	40	10	72	.55	.29	24.4	35.4	12.2
11	1	–	–	1–3	3–1	22	80	78	180	.55	.21	23.7	80.3	76.0
5	2	–	–	1–3	3–1	15	38	19	72	.67	.35	14.6	36.5	20.9
5	3, 4	–	–	1–3	3–1	10	25	1	36	.69	.41	11.8	17.8	6.4
Total 21						47	143	98	288	.589	.163	50.1	134.6	103.3

+/0 males in the absence of B/B females, rising to 59% in the presence of at least one of the latter.

A comprehensive test can be obtained by calculating the expected numbers of each kind of offspring from each population type in table 2 on this basis, omitting F_1. These calculations are summarized in the last 2 or 3 columns of tables 3 to 9. χ^2 was calculated in each case. A condensed summary is given below.

	Classes	DF	χ^2	Probability
Tables 3, 5, 6 (all V's = 1, $U_{B+} = 1$)	9	9	11.41	.20–.30
Tables 4, 7 (all V's = 1, $U_{BB} = .37$)	5	4	1.85	.70–.80
Table 8 (all V's = 1, $U_{B0} = .26$)	3	5	.53	ca .99
Table 9 (all V's = 1, $U_{BB} = .37$, $U_{B0} = .59$)	3	5	2.95	.70–80
	20	23	16.74	.80–90

It is obvious that no more complicated theory is necessary to account for the present data.

Rate of change of gene frequency per generation

The theoretical relation between gene frequencies in successive generations can be expressed in simple formulae with sufficient accuracy for most purposes in cases in which the selective differentials are all small. The present data are not among these cases. The distribution of gene frequencies in the offspring can, however, be tabulated for the parental groups with each possible gene frequency from 1/12 to 11/12. The mean change in gene frequency (Δq) and the variance of changes ($\sigma_{\delta q}^2$) can then be expressed as empirical functions of parental gene frequency.

Table 10 and fig. 1 give these and related statistics (excluding F_1). The observed values of Δq may be compared with those expected (c_1) on the hypothesis of no differential viability, $U_{BB} = .37$, $U_{B0} = .26$ in the absence of B/B females

TABLE 10. *Frequency of parental populations (generations 1 to 9) with each number of Bar genes, change of gene frequency (Δq) to next generation ratio, $\Delta q/q(1-q)$, variance ($\sigma_{\delta q}^2$) of frequencies in offspring generation and ratio $q(1-q)/\sigma_{\delta q}^2$.* The relative constancy of the ratio $\Delta q/q(1-q)$ indicates that selection pressure is empirically of the form $\Delta q = -.35q(1-q)$. The average of the ratio $q(1-q)/\sigma_{\delta q}^2$ indicates a value of $2N_e$ of 9.74. The calculated values c_1 are those expected from the vials, classified according to the number of Bar genes among the parents on the hypotheses of no differential viability, $U_{BB} = .37$, $U_{B0} = .26$ in absence of B/B females but .59 in their presence. The calculated c_2 are from the empirical formula $\Delta q = -.35q(1-q)$.

No. Bar genes	No. parents (gen.) (1–9)	q	Δq 0	c_1	c_2	$\frac{\Delta q}{q(1-q)}$	$\sigma_{\delta q}^2$	$\frac{q(1-q)}{\sigma_{\delta q}^2}$
1	84	.0833	−.0248	−.0209	−.0267	−.325	.0063	12.1
2	77	.1667	−.0314	−.0491	−.0486	−.226	.0141	9.9
3	88	.2500	−.0814	−.0738	−.0656	−.434	.0200	9.4
4	84	.3333	−.0724	−.0749	−.0778	−.326	.0226	9.9
5	63	.4167	−.0939	−.0879	−.0851	−.386	.0259	9.4
6	49	.5000	−.0884	−.1014	−.0875	−.354	.0348	7.2
7	21	.5833	−.0793	−.1060	−.0851	−.326	.0399	6.1
8	12	.6667	−.0903	−.1009	−.0778	−.406	.0119	18.6
9	10	.7500	−.1500	−.0947	−.0656	−.800	.0321	5.8
10	2	.8333	0	−.1116	−.0486	−.0	.0556	2.5
11	2	.9167	−.0417	−.0559	−.0267	−.546	.0313	2.4
	492					−.3511		9.74

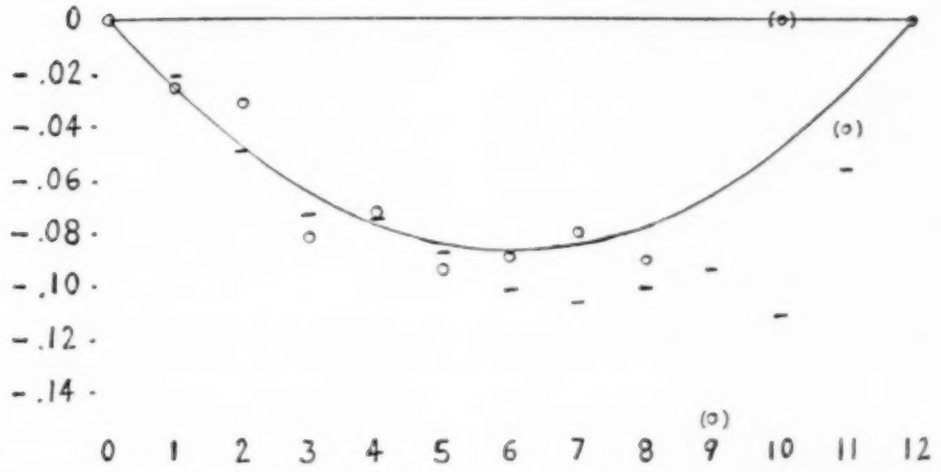

FIG. 1. The observed average rate of change in gene frequency per generation is shown in relation to the number of Bar genes per population by small circles. The rates of change, calculated on the hypothesis that $U_{BB} = .37$, $U_{BU} = .26$ in the absence of B/B females, .59 in their presence, are indicated by short lines. The rates of change calculated from the empirical formula $\Delta q = -.35q(1-q)$ are indicated by the smooth curve. Parentheses indicate observations based on highly inadequate numbers of cases.

but .59 in the presence of at least one B/B female. Calculations were made for every type of population in table 2. It may be seen that these theoretical values agree fairly well with those observed. A comparison of observed and calculated numbers of each genotype at each parental gene frequency (grouping 10/12 and 11/12) yields $\chi^2 = 17.5$ for females (about 16 degrees of freedom, probability .20–.30), $\chi^2 = 14.2$ for males (about 9 degrees of freedom, probability .10–.20), with a total χ^2 of 31.8, 25 degrees of freedom, probability .10–.20.

The observed values of Δq divided by $q(1-q)$ yield a series of values with weighted average $\bar{s} = -.351$ and only a slight trend ($s = -.344-.024q$). The simple empirical formula $\Delta q = -.35q(1-q)$ is adequate for most purposes.

The theoretical formula for selection in the simple case of an autosomal semidominant gene in a large population is $\Delta q = sq(1-q)$. The approach to this form in the present case in spite of the recessiveness of selective disadvantage in females ($U_{++} = U_{B+} = 1$, $U_{BB} = .37$) is due in part to the higher gene frequency in males than in females for most values of q which throws the point of maximum selection in males at values of q less than .50, and to some extent to the falling off of selection in males at high values of q due to the presence of B/B females.

The variance of gene frequencies takes the form $q(1-q)/2N_e$ where N_e is the effective size of population. Table 10 shows the ratios $q(1-q)/\sigma_{\delta q}^2$ as estimates of $2N_e$. There is considerable irregularity but no well defined trend. The weighted average is 9.74 to be compared with the theoretical value 13.53 in populations of 4 females, 4 males (with sex linkage, no selection and fluctuation due merely to accidents of sampling). The value indicated by the rate of fixation of forked and its type allele, with very little selection, was 11.22.

The distribution of gene frequencies

It is of interest to compare the observed distribution of gene frequencies after attainment of equilibrium of form with that expected under the observed selection pressure and observed effective size of population. We may safely use the model of a monoecious diploid population.

The class with gene frequency q contributes to the classes of the next generation according to the expansion of

$$[(1-q-\Delta q)(B^+)+(q+\Delta q)(B)]^{2N_e}f(q)$$

where f(q) is the frequency at gene frequency q.

The conditions for stability of form under fixation at the constant rate k can be represented as follows writing q and q_1 for the frequency of donor and recipient classes with respect to B, and $p = (1-q)$ and $p_1 = (1-q_1)$, and writing N here for effective size of population.

$$\frac{(2N)!}{(2Np_1)!(2Nq_1)!}\sum_{q=1/2N}^{1-1/2N}[(q+\Delta q)^{2Nq_1}(p-\Delta q)^{2Np_1}f(q)] = (1-k)f(q_1)$$

As brought out later, the form depends largely on the value of 2Ns (= −3.40). As it is convenient to use the integer 10 for 2N, s will be taken here as −.34.

$$\frac{20!}{(20p_1)!(20q_1)!}\Sigma(q-.34\,pq)^{20q_1}(p+.34\,pq)^{20p_1}f(q) = (1-k)f(q_1)$$

Starting from a population with gene frequency .50, the distribution for successive generations (total, including newly fixed classes, 1.0000) is given in table 11 for generations 1 to 10, generation 13 in which fixation of type reaches its maximum and for an indefinitely large number of generations. The distribution is sufficiently close to final form from generations 4 to 10 to give an appearance of uniformity with such numbers as were available in our data.

The limiting rates of fixation of type and Bar come out 20.2% and 0.75% in good agreement with the observed 22.0% and 0.7%.

Unfortunately the calculated frequencies for the unfixed classes cannot be compared directly with the observed distribution because of the difference in number of classes. It may be shown, however, that the goodness of fit depends much more on 2Ns than on N itself by considering the extreme case in which N is indefinitely large but 2Ns is still −3.40.

The condition for stability of form in this limiting case can be represented sufficiently accurately by replacing summation by integration, f(q) by $\varphi(q)/2N$ or $\varphi(q)dq$, and $(x)! = \Gamma(x+1) = x\Gamma(x)$

$$\frac{\Gamma(2N)}{p_1q_1\Gamma(2Np_1)\Gamma(2Nq_1)}\int_0^1(q+\Delta q)^{2Nq_1}(p-\Delta q)^{2Np_1}\varphi(q)\,dq = (1-k)\varphi(q_1)$$

TABLE 11. *The theoretical distribution of gene frequencies among populations in which 2N = 10, $\Delta q = -.34q(1-q)$, in successive generations, starting from q = .50.* The entries in rows 0 and 10 represent the frequencies of newly fixed populations. Fixation reaches a peak in generation 13, close to the limiting rate. The last column gives estimates from the distribution for 2N = ∞, 2Ns = −3.4.

No. of Bar genes	Generation 2N = 10, 2Ns = −3.4										2N = ∞ 2Ns = −3.4
	0	1	2	3	4	6	8	10	13	∞	
0		.0047	.0601	.1240	.1610	.1911	.1998	.2021	.2026	.2019	.2066
1		.0333	.1280	.1813	.2064	.2252	.2304	.2317	.2318	.2311	.2602
2		.1063	.1734	.1798	.1782	.1755	.1743	.1738	.1735	.1731	.1704
3		.2011	.1846	.1558	.1396	.1263	.1223	.1210	.1205	.1204	.1142
4		.2497	.1646	.1241	.1051	.0905	.0862	.0850	.0846	.0847	.0783
5	1.0000	.2125	.1260	.0918	.0765	.0650	.0618	.0609	.0607	.0610	.0551
6		.1256	.0833	.0632	.0538	.0468	.0449	.0444	.0444	.0448	.0397
7		.0509	.0473	.0404	.0365	.0336	.0330	.0330	.0331	.0335	.0293
8		.0136	.0224	.0236	.0237	.0239	.0241	.0244	.0246	.0250	.0222
9		.0021	.0083	.0119	.0138	.0155	.0162	.0165	.0168	.0170	.0171
10		.0002	.0020	.0041	.0054	.0066	.0070	.0072	.0074	.0075	.0069
Tot.		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

The solution for selection pressures of the fairly general form $\Delta q = (s + tq) \times q(1-q)$, which allows for any degree of dominance, has been given in powers of s and t jointly, for very small k (Wright, 1942). It would be very tedious to solve it in this way for nonnegligible k since the value of k in relation to s and t must be established by repeated trial. Fortunately for our present purpose, the case, $\Delta q = spq$, is much simpler. The method used in a previous paper (Wright, 1931) leads at once to a solution in the form

$$\varphi(q) = e^{2Nsq}(a_0 + a_1 q + a_2 q^2 + a_3 q^3 \cdots)$$

in which s appears in the a's only in even powers, after dropping terms in which the exponent of N is less than that of s. Thus the value of the power series remains the same on reversing the sign of the selection. As the distribution must remain the same in the case $\Delta q = sq(1-q)$ on reversing the sign of s and replacing q by $(1-q)$, it is evident that a series of the form

$$\varphi(q) = Ce^{2Nsq}(1 + C_1 pq + C_2 (pq)^2 + C_3 (pq)^3 \cdots)$$

can be used to advantage. In this case the coefficients came out as follows to a sufficient approximation.

$$C_1 = (1 - 2Nk)$$
$$C_2 = \tfrac{1}{3}[6 - 2Nk)C_1 + 2N^2 s^2]$$
$$C_3 = \tfrac{1}{6}[(15 - 2Nk)C_2 + 2N^2 s^2 C_1]$$
$$C_n = \frac{2}{n(n+1)}\{[n(2n-1) - 2Nk] \times C_{n-1} + 2N^2 s^2 C_{n-2}\}$$

The same result can be obtained from the Focker-Planck equation

$$\frac{1}{2}\frac{\partial^2}{\partial q^2}[\sigma_{\delta q}{}^2 \varphi(q, t)] - \frac{\partial}{\partial q}[\Delta q \varphi(q, t)] = \frac{\partial \varphi(q, t)}{\partial t}$$

in the form appropriate to constancy of form (excluding the fixed classes) with decay at the rate k per generation (Wright 1945).

$$\frac{1}{2}\frac{d^2}{dq^2}[\sigma_{\delta q}{}^2 \varphi(q)] - \frac{d}{dq}[\Delta q \varphi(q)] + k\varphi(q) = 0$$

For $\sigma_{\delta q}{}^2 = q(1-q)/2N$, $\Delta q = sq(1-q)$ this reduces to

$$\frac{d^2}{dq^2}[q(1-q)\varphi(q)] - 4N\frac{d}{dq}[q(1-q)\varphi(q)] + 4Nk\varphi(q)$$

On substituting $\varphi(q) = e^{2Nsq}\chi(q)$ we get after some reduction

$$\frac{d^2\chi(q)}{dq^2} - \left[4N^2 s^2 - \frac{4Nk}{q(1-q)}\right]\chi(q) = 0$$

If

$$\chi(q) = [1 + C_1 q(1-q) + C_2 q^2 (1-q)^2 \cdots]$$

as assumed above, we are led to the same relations among the coefficients as by the other method in this case without dropping small terms, as approximation is already introduced in the use of the Focker-Planck equation.

After $n(2n-1)$ has become greater than $2Nk$, the first term in C_n has the same sign as C_{n-1}. The second always has the sign of C_{n-2}. Thus if two successive coefficients (after this point) ever have the same sign all later ones must have the same sign and will soon begin to increase. The series of coefficients becomes either divergent positive or negative unless there is a relation of $2Nk$ to $2N^2 s^2$ at which the coefficients continue to alternate in sign and approach zero. The rapidity of divergence, one way or the other, is so great that it is easy to locate to any required number of decimal places the desired value of $2Nk$. This may be taken to be the limiting rate of fixation under the assumed selection. The final steps in the present case were as follows:

Trial values of 2Nk

	2.13536800	2.13536802	2.13536810
C_1	−1.13536800	−1.13536802	−1.13536810
C_2	+ .46407350	+ .46407348	+ .46407341
C_3	− .09871538	− .09871544	− .09871568
C_4	+ .01291079	+ .01291063	+ .01290997
C_5	− .0011439	− .0011444	− .0011464
C_6	+ .0000747	+ .0000732	+ .0000674
C_7	+ .0000011	− .0000039	− .0000239
C_8	+ .0000154	− .0000012	− .0000674

A number of trials led to the value 2.135368 for 2Nk. Using this, the coefficients alternate in sign to C_6 but thereafter all are positive and are divergent (after C_7). This shows that this estimate of 2Nk is too small. On trying 2.1353681 the signs alternate down to C_7 but are all negative thereafter, showing that this estimate is too large. Interpolation suggests 2.13536802. This also turns out to be too large but is correct for 9 significant figures.

It has been found empirically that for values of 2Ns up to 1, 2Nk is given to 11 significant figures by

$$2Nk = 1 + \frac{(2Ns)^2}{10} - \frac{(2Ns)^4}{7000} - \frac{(2Ns)^6}{1{,}050{,}000} - .000{,}000{,}004(2Ns)^8$$

This is accurate to 6 significant figures up to 2Ns = 4, to 3 significant figures for 2Ns = 6.

It is desirable to determine whether the value of 2Nk, determined above, is consistent with the rates of fixation of the two alleles for given 2N, deducible from the terminal ordinates of the distribution.

In the absence of selection, the formula for the distribution curve reduces at once to $\varphi(q) = 1$ or $f(q) = 1/(2N)$. The chance of fixation in a population with r representatives of the specified allele is e^{-r} (from the Poisson Law). The total amount of fixation of this allele should thus be

$$\frac{1}{2N}(e^{-1} + e^{-2} + e^{-3} \cdots) = .582/(2N)$$

if all classes have the frequency $1/(2N)$ (Wright, 1931). As the actual rate is exactly $1/(4N)$ (in the ideal case of completely random union among 2N gametes) the above approximate formula for the curve must be considerably modified near the terminal ordinates. The correction to be applied to the approximate formula $\varphi(q) = 1$ was determined by Fisher (1930) on inspection of the above paper in manuscript: .8182 for the class with one of the genes, .9168 for that with two, .9449 for that with three, .9583 for that with four and thereafter $1 - [1/(6r)]$.

If selection is present and 2N is indefinitely large, the frequencies from the approximate formula

$$f(q) = Ce^{2Nsq}(1 + C_1pq + C_2(pq)^2 \cdots]/(2N)$$

take the value $C/(2N)$ in the neighborhood of $q = 0$ and the value $Ce^{2Ns}/(2N)$ in the neighborhood of $q = 1$. Because of the approximate uniformity in each of these neighborhoods, corrections closely similar to those determined by Fisher for the case of no selection should be applicable.

Fixation occurs practically from only a very few of the classes in these terminal regions. Again because of the approximate uniformity of the frequencies in each region,

$$k_0 = f(0)/(2N) = C/(4N)$$
$$k_1 = f(1)/(2N) = Ce^{2Ns}/(4N)$$
$$k = C(1 + e^{2Ns})/(4N)$$

Thus

$$C = 4Nk/(1 + e^{2Ns})$$
$$f(q) = 2ke^{2Nsq}(1 + C_1pq + C_2(pq)^2 \cdots)/(1 + e^{2Ns})$$

Let $y = e^{2Nsq}(1 + C_1pq + C_2(pq)^2 \cdots)$ represent the relative value of an ordinate calculated from 2Ns and the values of the C's given above. The ordinates of the curve of unit area can be obtained from the relation $\varphi(q) = y/\int_0^1 y\,dq$. If values of y are calculated at uniform intervals from $q = 0$ to $q = 1$, the area

of the curve can be estimated from a quadrature formula. The following were obtained from ordinates at intervals 0.1.

Quadrature rule

	Trapezoidal	Parabolic (Simpson's)	Gregory's	Correct values
$2Nk_0$	2.0353	2.0659	2.06639	2.066,406
$2Nk_1$	.0679	.0689	.06896	.068,962
$2Nk$	2.1032	2.1347	2.13535	2.135,368

It may be seen that the trapezoidal quadrature gives estimates of 2Nk that agree with the actual values to two significant figures, Simpson's rule to four and Gregory's to at least five. The estimate of 2Nk derived from the criterion of convergence is thus consistent with the values of the terminal ordinates.

As the values $k_0 = k_1 = 1/(4N)$ apply exactly in the absence of selection for all values of 2N, however small (in the ideal case of completely random union of gametes), it may be surmised that the formulae given above as applying in the presence of selection to very large populations will give fairly good approximations for small 2N. In the case $2N_e = 10$, we obtain $k_0 = .2066$, $k_1 = .0069$ in comparison with .2019 and .0075 respectively, the figures from direct determination of the stable distribution for $2N = 10$, $2Ns = -3.4$. The estimates for the frequencies of the unfixed classes (by adjusting the relative ordinates at intervals of 0.1 to give the total, $1 - k_0 - k_1$) are more seriously in error (table 11) and need correction of the type referred to.

The agreement in form for $2N = 10$ (direct) and $2N = \infty$ (no correction), both for $2Ns = -3.4$, is, however, sufficiently close that it may be surmised that the form for $2N = 12$, $2Ns = -3.4$, determined directly, should be very close to that for $2N = 10$ and the same 2Ns and thus will permit good estimates for the unfixed classes (1/12 Bar to 11/12 Bar) even though the effective value of 2N is about 10 instead of 12. The actual values are given in the second column of table 12 in comparison with uncorrected estimates from the appropriate ordinates for $2N = \infty$. We note again that the rates of fixation agree

TABLE 12. *The theoretical distribution of gene frequencies, including newly fixed populations, after stability of form has been attained.* Column 2: exact determinations for $2N = 12$, $2Ns = -3.4$. Column 3: estimates of same from limiting curve. Column 4: newly fixed populations from $2N = 10$, $2Ns = -3.4$, unfixed populations rated down from Column 2. Column 5: estimates of same from limiting curve. Column 6: observed populations. Column 7: expectations calculated from Column 4. Column 8: difference ($\chi^2 = 8.9$). Column 9: expectations calculated for Column 5. Column 10: difference ($\chi^2 = 11.7$).

	$2Ns = -3.4$								
	$2N_e = 12$		$2N_e = 10$						
	Direct	Estimate from $2N = \infty$	Direct	Estimate from $2N = \infty$	Obs.	Calc. $2N_e = 10$ (Direct)	Diff.	Calc. $2N_e = 10$ from $2N = \infty$	Diff.
0	.1689	.1722	(.2019)	(.2066)	65	59.6	+5.4	61.0	+4.0
1	.2059	.2346	.1973	.2244	57	58.2	−1.2	66.2	−9.2
2	.1647	.1642	.1579	.1571	42	46.6	−4.6	46.3	−4.3
3	.1214	.1167	.1163	.1116	35	34.3	+0.7	32.9	+2.1
4	.0894	.0842	.0857	.0806	27	25.3	+1.7	23.8	+3.2
5	.0668	.0619	.0640	.0592	20	18.9	+1.1	17.5	+2.5
6	.0507	.0462	.0486	.0442	19	14.3	+4.7	13.0	+6.0
7	.0391	.0351	.0375	.0336	13	11.1	+1.9	9.9	+3.1
8	.0306	.0271	.0293	.0259	5	8.6	−3.6	7.7	−2.7
9	.0241	.0213	.0231	.0204	7	6.8	+0.2	6.0	+1.0
10	.0188	.0170	.0180	.0163	1	5.3	−4.3	4.8	−3.8
11	.0134	.0138	.0129	.0132	2	3.8	−1.8	3.9	−1.9
12	.0062	.0057	(.0075)	(.0069)	2	2.2	−0.2	2.0	0.0
	1.0000	1.0000	1.0000	1.0000	295	295.0	0.0	295.0	0.0

much better than the frequencies of the unfixed classes.

The forms of these distributions can be compared by plotting the frequencies for $2N = 12$ on a percentage scale in which the units are 20% larger than those used in plotting the distribution for $2N = 10$ (abscissas 0–12 in the former, 0–10 in the latter) and by plotting the curve for $2N = \infty$ in such a way that it has a unit area over the base 2N on the appropriate scale of percentages whether $2N = 10$ or 12. It may be seen that the forms for $2N = 10$ and $2N = 12$

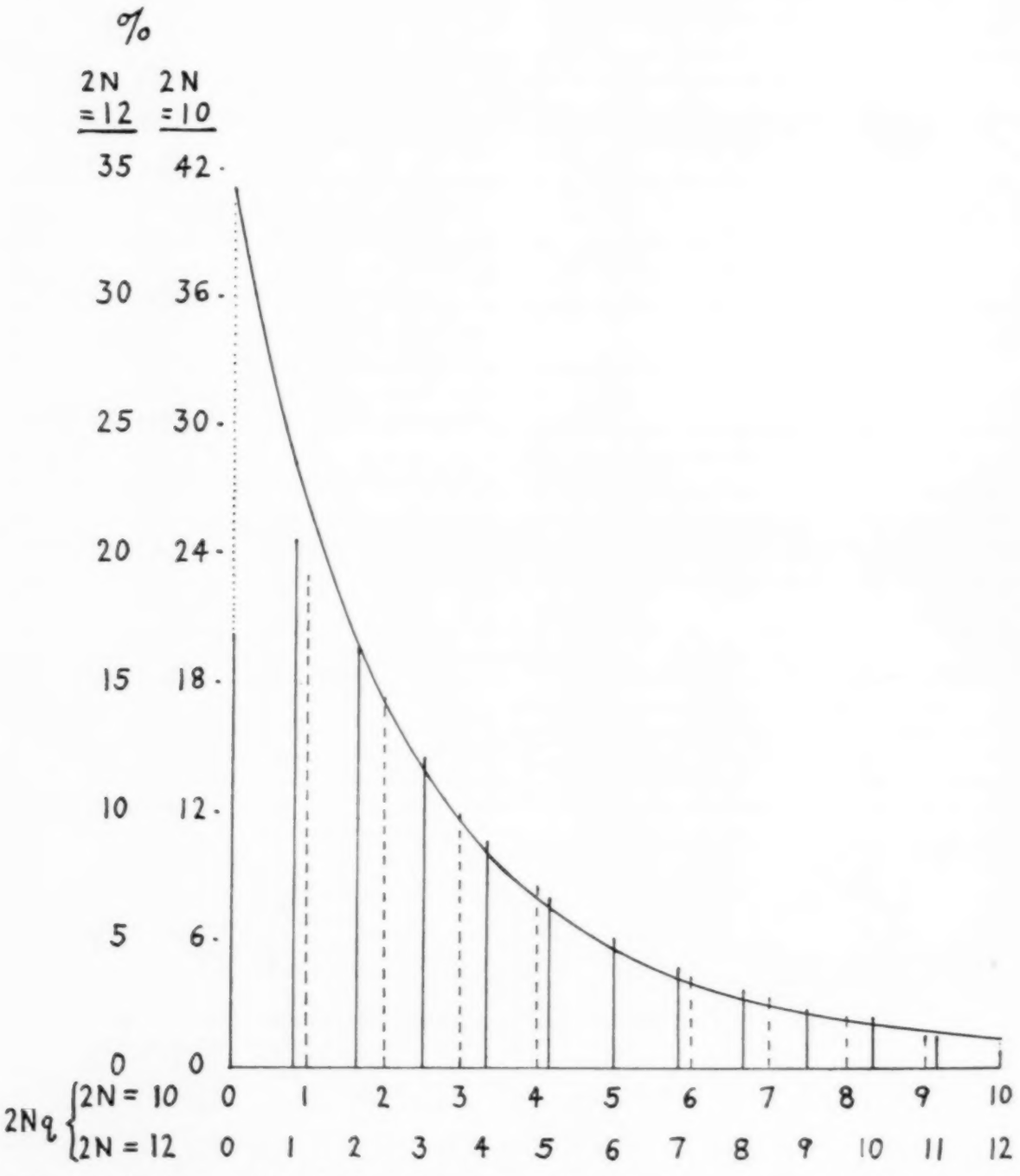

FIG. 2. The theoretical distributions of gene frequencies, including the classes of newly fixed genes, after attainment of stability of form, among populations in which $2Ns = -3.4$, $2N = 12$ (solid lines), $2N = 10$ (broken lines, except at $q = 0$, .5 and 1.0 at which there is agreement with the values for $2N = 12$ on the scales used). The scale unit for percentage frequencies is 20% greater for $2N = 12$ than for $2N = 10$ to bring out the similarity of form. The distribution for $2N = \infty$ is shown as a smooth curve of unit area (base 10 or 12, each with the appropriate scale of ordinates).

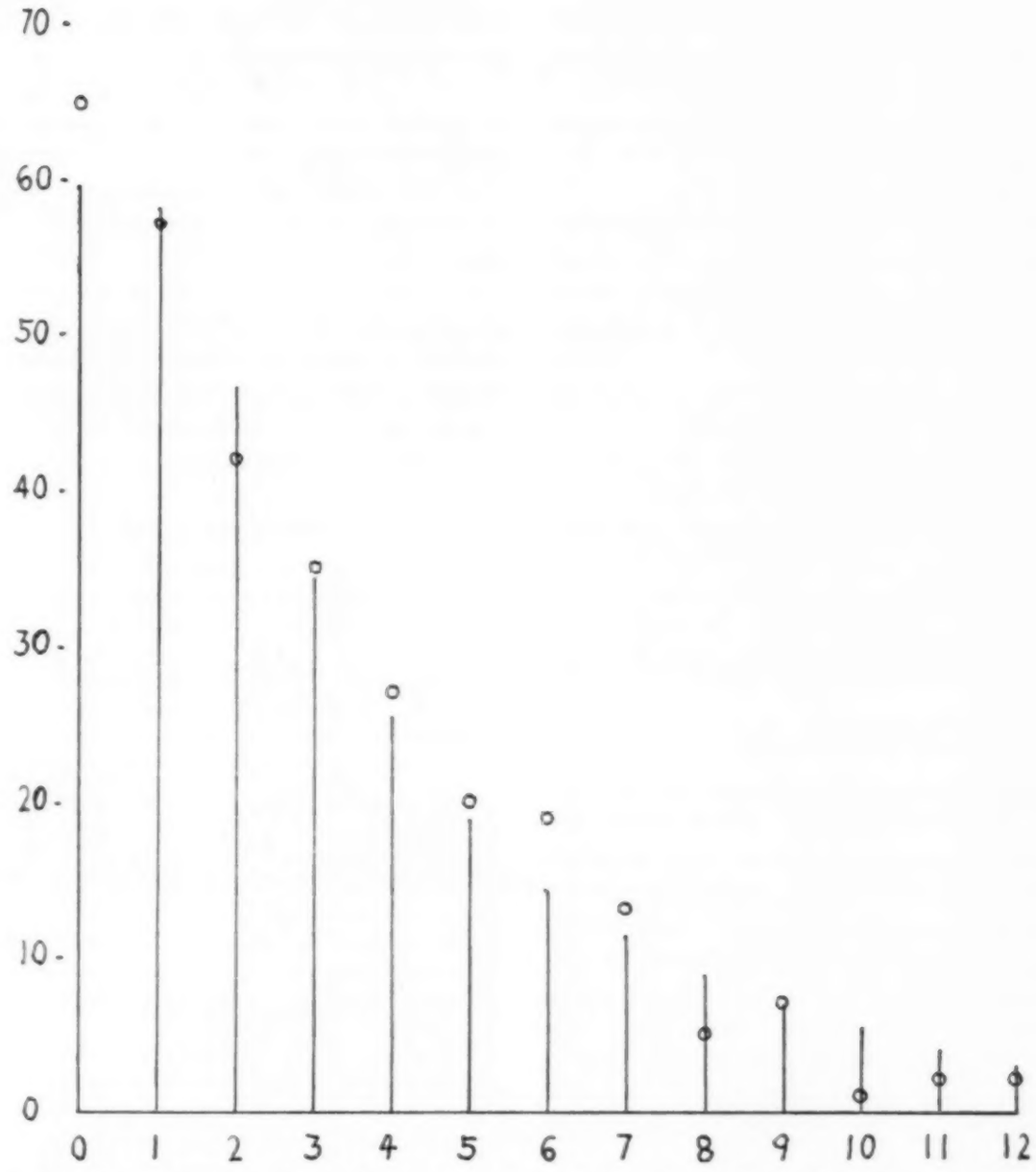

FIG. 3. The observed number of populations containing one to eleven Bar genes or newly fixed with respect to type (0) or Bar (12) in generations 4 to 10 (small circles). The expected numbers are indicated by vertical lines. Those for the newly fixed classes are based on the theoretical percentages of fixation for $2N = 10$, $2Ns = -3.4$. Those for the unfixed classes apportion the remainder according to the theoretical frequencies for $2N = 12$, $2Ns = -3.4$.

agree very closely, that for $2N = \infty$ less closely.

To test the goodness of fit of the experimental frequencies, the expected numbers of newly fixed classes are deduced from the distribution for $2N = 10$, while those for the unfixed are deduced from the relative frequencies of those for $2N = 12$. The expected proportions are shown in the fourth column of table 12. Similar estimates from the curve $2N = \infty$ (uncorrected) are given in the next column. The observed distribution of newly fixed and unfixed populations in generations 4 to 10, combined, are shown in column 6 and the expected values, rated up to the same total, 295, in column 7 and 9. The differences are given in columns 8 and 10. Calculation of χ^2 for the observed and direct estimates gives 8.9 with 10 degrees of freedom, probability .50–.70. Even the uncorrected estimates from the ordinates of the curve for $2N = \infty$ gives a χ^2 of only

11.7, probability about .30. The estimated rates of fixation from the latter, taking $2N_e = 9.74$ instead of 10 come out 21.2% for k_0 and 0.7% for k_1 in comparison with observed 22.0% and 0.7% (cf. fig. 3).

The calculation of the exact distributions for values of 2N and $2N_e$ much larger than 12 and 10 respectively would be a very tedious matter. Estimates from the curve for $2N = \infty$, observed 2Ns, become increasingly satisfactory as the observed 2N and $2N_e$ become larger. From the goodness of fit in the present case, it appears that the method should give comparisons of observation and theory for larger populations, that are adequate for most purposes, especially if the corrections, derived from the case of no selection, are applied to the subterminal classes.

Summary

Experiments with 108 very small populations of *Drosophila melanogaster* involving the mutation Bar and its allele (four females and four males as parents of each generation), carried to fixation of one allele or to the tenth generation, indicated little selective mortality, but severe selection against Bar from low productivity of homozygous Bar females (37% of that of B/+ or +/+) and Bar males (26% of the +/0 in the absence of B/B females, 59% in their presence).

Starting from 50% Bar genes in each case, the distribution of frequencies soon reached approximate stability of form (about four generations) as type came to be fixed at a rate of 22.0% per generation and Bar at a rate of 0.7% per generation. After generation 10, type had been fixed in 95 lines, Bar in three and 10 were still unfixed.

The form of the distribution agreed well with that expected in a population of effective size 72% of the actual size and an empirically determined rate of change of the frequency (q) of the Bar gene, $\Delta q = -.35q(1 - q)$. The limiting form of the distribution curve for indefinitely large populations, but the observed value of 2Ns, is derived, and shown to give a basis for fairly good estimates even with such small populations as in these experiments.

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INTEGRATION OF THE GENOTYPE IN LOCAL POPULATIONS OF THREE SPECIES OF *DROSOPHILA*

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Received February 8, 1954

INTRODUCTION

Recent work on genetics of natural populations has shown that the adaptedness of most "normal" individuals found in natural habitats is due to a state of heterosis. In at least some sexually reproducing and cross-fertilizing species, most individuals are heterozygotes for gene complexes which are distinctly deleterious if present in double dose. However, these gene complexes are so mutually adjusted, or coadapted, by natural selection in the process of evolution that the heterozygotes possess high fitness (Dobzhansky and Spassky, 1953; Wallace and Madden, 1953). The gene pool of the population of any one geographic region contains, then, a variety of coadapted gene complexes. Any of these gene complexes occurs only rarely in homozygous condition, and the fitness of the population depends upon its being composed chiefly of heterotic heterozygotes. Natural selection does not, however, adjust the gene complexes in different geographic populations to produce highly fit heterozygotes, for the simple reason that interbreeding of members of geographically remote populations occurs only rarely or not at all. A comparison of the genotypic structure of geographically isolated populations becomes, then, important.

The present article reports the results of experiments on geographic populations of three species of *Drosophila: D. pseudoobscura, D. willistoni,* and *D. paulistorum.* In each of these species the fitness of three or more populations derived from geographically remote localities was examined under highly artificial but carefully controlled experimental conditions. Next the fitness of the F_1 and F_2 hybrids between these populations was examined and compared with that of the parental races. The experiments showed that the F_1 hybrids displayed in most instances a significant increase of the viability; this increase was not only lost in the F_2 generation, but the F_2 hybrids were usually inferior to the parental races. The genotype of each local population is, in at least some sexually reproducing species, an integrated system which may break down as a result of gene recombination in the hybrids.

MATERIAL AND TECHNIQUE

The material on *Drosophila-pseudoobscura* Frolowa consisted of eight strains, each derived from a single impregnated female caught in nature, from each of the following five localities (40 strains in all [1]): Piñon Flats, on Mount San Jacinto, in California; Mather, Sierra Nevada, California; Bryce National Park, Utah; Ferron Canyon, Utah; and Black Canyon of the Gunnison National Monument, Colorado. The Mather, Bryce, Ferron and Gunnison strains were collected by Professor Th. Dobzhansky during the summer of 1950. The Piñon strains were obtained by Dr. Donald Mitchell, who collected them in 1949. All strains were homozygous for the Arrowhead gene arrangement in their third chromosomes. Thus, the structural heterozygosis was not involved at all in the experiments to be described.

D. willistoni Sturtevant and *D. pauli-*

[1] In the previous description of the experiments (Vetukhiv, 1953) it has been erroneously stated that 20 strains, instead of 40, have been used.

storum Dobzhansky and Pavan were studied from three localities in Brazil: Belem, state of Para, Içana on the upper Rio Negro, just north of the Equator, state of Amazonas, and Cantareira, near the city of São Paulo. The flies were collected at Belem and at Içana by Professor Dobzhansky, and at São Paulo by Professor A. Brito da Cunha in 1951. Ten strains of each species from each of the three localities were available (60 strains in all).

The three following types of crosses were made for each species: between strains derived from the same locality, between strains derived from different localities, and between the F_1 hybrids. In all crosses, mixtures of females from some and males from other strains from the localities named were used, so that the eggs obtained represented populations of known geographic origin.

The parent flies were placed for 24 hours in glass vials containing paper spoons with the regular cream-of-wheat and molasses medium. Freshly hatched larvae were transferred into vials containing 4 cc. of the synthetic food medium described by Kalmus (1943), except that the amount of agar was doubled. On the surface of the medium was placed 0.1 cc. of freshly prepared 14 per cent suspension of dry Fleischmann's yeast. In the experiments with *D. pseudoobscura*, 10 freshly hatched larvae of the wild-type populations to be studied, and 10 freshly hatched larvae of the control mutant (orange eye) were placed in every vial. Each vial contained, thus, 20 larvae. In the experiments with *D. willistoni* and *D. paulistorum* 20 larvae from the wild populations were placed in each vial, and to them were added 20 larvae of the mutant white eye of *D. willistoni*. The white mutant served, of course, as a control. All vials were left at 25° C. until the adult flies hatched. The numbers of the mutant and the wild-type individuals among the flies hatching in each vial were recorded.

The amount of food placed in each vial was made deliberately insufficient for the growth and development of 20 larvae of *D. pseudoobscura*, or of 40 larvae of *D. willistoni* or *D. paulistorum*. The number of the adult flies of the first named species hatching per vial was 8.8, and for *D. willistoni* and *D. paulistorum* was 18.6 and 17.4. Moreover, the larvae of the orange-eyed and the white-eyed mutants are weaker than the wild-type of the respective species. The proportions of the larvae surviving to become adult flies can be used as indices of the viability of the larvae of a given genotype under conditions of fairly strenuous competition in overpopulated cultures. The presence of mutant larvae served as a control of a

TABLE 1. *Viability (per cent survival) in local populations of* Drosophila pseudoobscura *and their hybrids*

Localities	Number	Survival % Experimental	Survival % Control
Mather	350	60.3	30.0
Bryce	360	60.0	27.9
Gunnison	340	54.7	25.9
Mather × Bryce F_1	360	72.2	32.5
Mather × Gunnison F_1	360	66.1	30.3
Gunnison × Bryce F_1	360	69.7	26.4
Piñon	220	54.1	31.3
Bryce	240	69.5	31.7
Ferron	230	62.1	34.7
Gunnison	240	56.2	26.6
Piñon × Bryce F_1	220	79.8	28.1
Piñon × Ferron F_1	230	77.8	25.6
Piñon × Gunnison F_1	220	69.3	30.0
Ferron × Gunnison F_1	240	57.7	32.5
Mather	500	62.0	29.0
Bryce	520	54.0	23.7
Gunnison	520	50.4	30.0
Mather × Bryce F_2	510	57.8	28.9
Gunnison × Mather F_2	520	41.9	25.9
Gunnison × Bryce F_2	540	43.0	29.8
Piñon	210	52.4	27.6
Bryce	180	66.1	35.5
Ferron	180	59.4	34.4
Gunnison	200	71.6	35.5
Piñon × Bryce F_2	200	47.0	26.5
Piñon × Ferron F_2	200	43.5	24.0
Piñon × Gunnison F_2	200	52.5	30.0
Ferron × Gunnison F_2	190	58.9	30.5

reasonable uniformity of the culture conditions in different experiments. The mutants are weaker than wild-type flies, and they die out if the conditions become too rigorous.

Experiments with *Drosophila pseudoobscura*

In the first series of experiments, conducted in January-March, 1952, the populations from Mather, Bryce, and Gunnison were studied, together with their F_1 and F_2 hybrids. The results are shown in table 1. The number of larvae in each experiment, as well as the percentage of survival to the adult stage are indicated. The numbers of the replications made (i.e., of the vials with larvae) can be obtained by dividing the numbers of the larvae by 10. As stated above, equal numbers of wild and of control larvae were used throughout. It can be seen that the percentages of survival of F_1 hybrid larvae are greater than those of survival of the larvae of the geographic races themselves. Thus, about 60 per

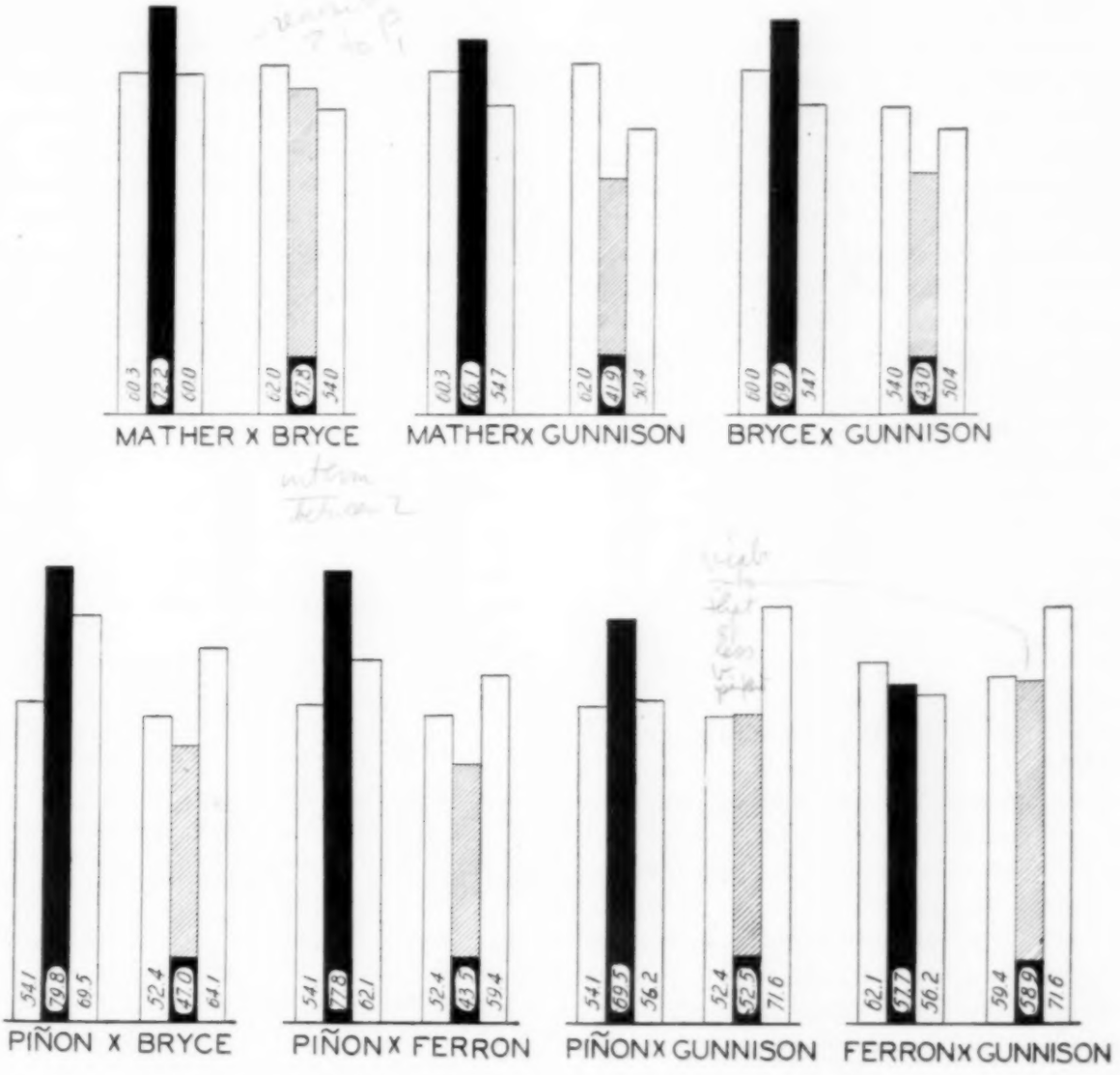

Fig. 1. Viability of geographic populations of *Drosophila pseudoobscura* and of hybrids between them. The heights of the white columns symbolize the viabilities of the parental populations, the black columns of the F_1 hybrids, and the cross-hatched columns of the F_2 hybrids between their populations. In every instance the parental populations were tested simultaneously with the F_1 hybrids, and re-tested simultaneously with the F_2 hybrids. The numerals indicate the per cent of survivals.

cent of Mather and Bryce, and about 55 per cent of Gunnison larvae, survive to become adults. This compares with the approximately 72 per cent survival of the Mather × Bryce, 66 per cent of Mather × Gunnison, and 70 per cent of Gunnison × Bryce F_1 hybrid larvae. This can be seen even more easily in figure 1, in which the height of the black columns symbolizes the percentages of the survival of the F_1 larvae. The black columns are placed between two white columns, the heights of which symbolize the survival percentages of the two ancestral populations.

The situation became, however, reversed in the F_2 hybrids, as shown in table 1 and figure 1. The parental populations, which were tested again, simultaneously with the F_2 hybrids, showed percentages of survival of approximately 62, 54, and 50 for Mather, Bryce and Gunnison respectively. The F_2 hybrids showed 58, 42, and 43 per cent survival for Mather × Bryce, Gunnison × Mather, and Gunnison × Bryce respectively.

A second series of experiments was conducted in July-September of 1952, this time using the populations from Piñon, Bryce, Ferron and Gunnison. The results are reported in table 1 and figure 1; in this figure the column the height of which symbolizes the hybrids is always placed between the columns which symbolize the viability of the parental races, as determined in tests conducted simultaneously with the hybrids. It can be seen that the F_1 hybrids show viabilities superior to their parents, except for Ferron × Gunnison which are intermediate between the parental races. In the F_2 generation the situation becomes reversed: the hybrids are now less viable than are the parental races. The Ferron × Gunnison hybrids are again exceptional, since their viability equals that of the less viable parental race (Ferron). It may be noted in this connection that the distance between the localities of Ferron and Gunnison is much smaller than those between Piñon (in California) on one side and Bryce, Ferron (Utah), and Gunnison (Colorado) on the other.

Experiments with *Drosophila willistoni*

These experiments were conducted during the winter 1952–1953. The results are reported in table 2 and figure 2. Since 20 larvae of the wild-type and 20 larvae of the control mutant were placed in each vial, the numbers of replicate experiments made are equal to the numbers of larvae shown in table 2, divided by 20.

Of the three F_1 hybrids, the ones between Belem and Içana show viabilities about equal to that of the more viable parent. The two other hybrids seem to display heterosis. In the F_2 generation, Belem × Içana suffers a breakdown of the viability, which is now clearly below that in either parental race. The other two hybrids loose in the F_2 the heterosis which they displayed in the F_1, but still have a viability about equal to that of the parental races.

Experiments with *Drosophila paulistorum*

Experiments with this species were first made in spring of 1953. The results obtained differed from those found in the analogous experiments with *D. pseudo-*

Table 2. *Viability (per cent survival) in local populations of* Drosophila willistoni *and their hybrids*

Localities	Number	Survival % Experimental	Survival % Control
Belem	360	58.0	35.8
Içana	340	63.2	33.5
São Paulo	340	58.5	26.8
Belem × Içana F_1	360	62.5	30.8
Belem × São Paulo F_1	360	69.7	36.9
São Paulo × Içana F_1	360	72.8	33.6
Belem	360	51.1	32.8
Içana	360	63.3	30.3
São Paulo	360	57.2	37.8
Belem × Içana F_2	360	41.9	37.2
Belem × São Paulo F_2	340	51.8	38.8
São Paulo × Içana F_2	360	60.8	29.2

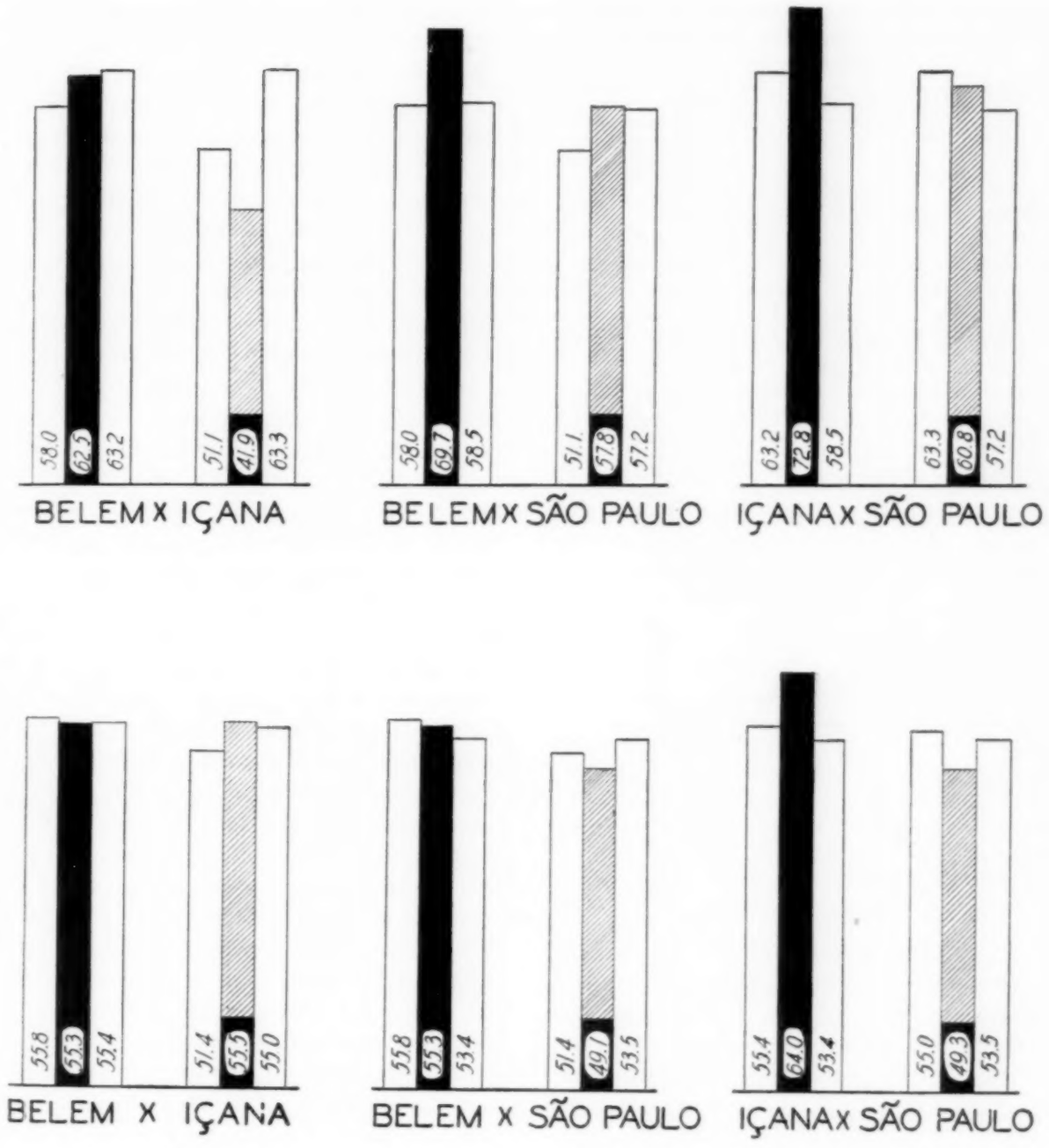

FIG. 2. Viability of the geographic populations and of hybrids between them in *Drosophila willistoni* (above) and in *Drosophila paulistorum* (below). The symbols are like those in figure 1.

obscura and *D. willistoni*. A repetition of the work with *D. paulistorum* was consequently judged desirable, and it was made during the autumn of 1953. The outcome of the first experiments was confirmed, as shown in table 3 and in figure 2.

In *D. paulistorum*, only the cross São Paulo × Içana displays a heterosis in the F_1 generation. The other two crosses, Belem × São Paulo and Belem × Içana, give F_1 hybrids which do not significantly differ from their parents in viability. The lack of heterosis in the F_1 seem to be correlated with an absence of a breakdown of the viability in the F_2 generation. Only in the first experiment, the F_2 hybrids of the cross Belem × São Paulo seem to show a lower viability (43.8 per cent) than either parental race (49.4 and

TABLE 3. *Viability (per cent survival) in local populations of* Drosophila paulistorum *and their hybrids*

Localities	Number	Survival % Experimental	Survival % Control
Belem	360	56.7	33.3
Içana	340	54.4	36.2
São Paulo	320	45.6	30.9
Belem × Içana F_1	300	54.2	35.3
Belem × São Paulo F_1	340	50.2	30.3
São Paulo × Içana F_1	320	64.4	41.8
Belem	360	55.2	33.3
Içana	360	56.3	28.6
São Paulo	360	60.2	30.2
Belem × Içana F_1	360	55.9	32.7
Belem × São Paulo F_1	360	59.8	31.1
São Paulo × Içana F_1	360	63.7	31.7
Belem	340	49.4	34.4
Içana	360	56.7	30.8
São Paulo	360	53.9	33.6
Belem × Içana F_2	360	55.3	38.1
Belem × São Paulo F_2	340	43.8	33.2
São Paulo × Içana F_2	320	52.2	31.6
Belem	440	53.0	26.4
Içana	440	53.6	31.8
São Paulo	440	52.1	31.6
Belem × Içana F_2	440	55.7	33.4
Belem × São Paulo F_2	440	53.2	31.6
São Paulo × Içana F_2	440	47.3	32.3

53.9 per cent respectively). The second experiment showed, however, no such difference between the hybrids and the parents. The heterosis observed in the F_1 of São Paulo × Içana does however disappear in the F_2 generation. To this extent, *D. paulistorum* behaves, then, like the two other species.

STATISTICAL TESTS

A statistical evaluation of the results obtained may begin with a consideration of the rates of survival of the control larvae. As stated in the chapter on Material and Techniques, every experimental vial with *D. pseudoobscura* contained 10 larvae of the mutant orange eye and 10 larvae of the type the viability of which was to be investigated. The experiments with *D. willistoni* and *D. paulistorum* had in each vial 20 larvae of the mutant white eye and 20 larvae of the genotype to be studied. The mutant larvae were, thus, a sort of a common denominator in all the experiments with a given species. The rates of survival of the mutants in the different experiments may then be taken as a measure of the adequacy of the control of the environmental conditions. With conditions becoming more rigorous, fewer mutant larvae should develop into adults then under conditions of less intense competition. Table 4 lists in the columns entitled "control" the chi-square values which characterize the degree of statistical significance of the differences between the rates of survival of the control larvae in the experiments indicated on the left of the table. All of these chi-squares have one degree of freedom. For one degree of freedom, a chi-square of 3.841 corresponds to the 5 per cent level of significance, and a chi-square of 6.635 to the one per cent significance level. Among the 60 chi-squares listed in table 4 for control experiments, only 5 have values above 6.635, and 6 have values between 3.841 and 6.635. Therefore, although the controls do not give quite uniform results, the rates of survival of the control larvae are reasonably close in the different experiments. In particular, there is no negative correlation observed between the rates of survival of the wild-type and of control (mutant) larvae in the same vial, as would be expected if the total number of survivors per vial were completely constant. On the contrary, there is an indication of a positive correlation between these survival rates. This suggests that whatever non-homogeneties were present in the experiments were due to uncontrolled variations in the quality of the food (particularly in the degree of stiffness of the agar).

The chi-square for the rates of survival of wild-type (experimental) larvae in the different experiments are given in table 4 in the columns "Experimental." These chi-squares also have one degree of freedom each. For *D. pseudoobscura*,

TABLE 4. *Chi-squares computed for various comparisons of local populations and their hybrids*

	F_1 Hybrids		F_2 Hybrids	
	Experimental	Control	Experimental	Control
Drosophila pseudoobscura				
Mather—Mather × Bryce	11.9	0.30	1.649	0.000
Mather—Gunnison × Mather	2.400	0.025	41.165	1.263
Bryce—Mather × Bryce	12.02	6.56	1.576	3.393
Bryce—Gunnison × Bryce	7.46	0.454	12.707	4.922
Gunnison—Gunnison × Mather	9.09	1.79	7.488	2.322
Gunnison—Gunnison × Bryce	16.02	0.027	6.066	0.000
Total (pure-hybrids)	27.38	2.67	19.78	0.158
Piñon—Piñon × Bryce	30.902	0.532	1.405	0.051
Piñon—Piñon × Ferron	29.671	1.512	3.167	2.592
Piñon—Piñon × Gunnison	9.310	0.096	0.000	0.190
Bryce—Piñon × Bryce	5.498	0.663	18.106	3.201
Ferron—Ferron × Gunnison	0.570	0.344	0.010	1.747
Ferron—Piñon × Ferron	13.414	4.546	9.505	11.676
Gunnison—Ferron × Gunnison	0.430	0.840	6.508	1.160
Gunnison—Gunnison × Piñon	8.245	0.687	14.490	1.374
Total (pure-hybrids)	22.231	0.857	22.039	7.612
Drosophila willistoni				
Belem—Belem × Içana	1.50	2.02	6.08	1.56
Belem—Belem × São Paulo	10.62	0.10	0.023	3.01
Içana—Belem × Içana	0.04	0.65	33.02	3.88
Içana—Içana × São Paulo	7.62	0.00	0.48	0.12
São Paulo—Belem × São Paulo	9.66	8.50	2.31	0.10
São Paulo—Içana × São Paulo	15.90	3.90	0.96	5.98
Total (pure-hybrids)	16.62	0.68	7.025	0.40
Drosophila paulistorum				
Belem—Belem × Içana	0.048	0.052	2.578	7.232
Belem—Belem × São Paulo	0.048	1.048	0.832	1.882
Içana—Belem × Içana	0.004	0.04	0.040	3.038
Içana—Içana × São Paulo	10.13	2.49	4.980	0.575
São Paulo—Belem × São Paulo	0.512	0.014	2.279	0.287
São Paulo—Içana × São Paulo	15.70	6.90	2.012	0.012
Total (pure-hybrids)	4.53	1.25	1.499	2.407

there are 16 chi-square in table 4 which pertain to the differences in the rates of survival of larvae of the parental populations and of the F_1 hybrid larvae. Of these, 12 chi-squares are significant at levels better then 1 per cent, one has a significance between the 5 and 1 per cent level, and 3 are not significant. As shown in table 1, the survival rates of F_1 larvae are higher than those of larvae of the parental races. For *D. willistoni* 5 out of the 7 chi-squares for the F_1 are significant, and 2 are not significant (table 4). In *D. willistoni* the F_1 hybrids tend to display heterosis. For *D. paulistorum* only 2 of the 7 chi-squares are significant at the 1 per cent level, and 1 (for the total) at between the 5 per cent and the 1 per cent level. In this species, heterosis does not necessarily occur in the hybrids between populations.

In contrast to the heterosis observed in most F_1 hybrids, the F_2 hybrids usually have a viability below that of the parental

races (tables 1–3). The chi-squares listed in table 4 in the column headed "F_2 Hybrids Experimental" are mostly significant in the experiments with *D. pseudoobscura* (in 10 out of the 16 cases the chi-squares are significant at the 5 per cent level or better). An analogous effect is indicated also for *D. willistoni* and *D. paulistorum* (compare tables 2, 3, and 4), but here only 4 out of the 14 chi-squares are significant at the same levels. A fact of some interest is an indicated negative correlation in *D. pseudoobscura* between the chi-square measuring the significance of the heterosis in the F_1 and the breakdown of the viability in the F_2 hybrids. In other words, the greater the heterosis displayed in the F_1 generation, the less pronounced the breakdown in F_2, and vice versa. A similar situation seems to obtain also in *D. willistoni*, but apparently not in *D. paulistorum*.

Perhaps more satisfactory for our material is the *t*-test of significance, which takes into consideration not only the difference of the totals for the different experiments but also the variance observed between the replicates (the vials) within each experiment. The values of *t* were computed according to the formula:

$$t = \frac{\sqrt{\frac{mn}{m+n}}\,|\bar{X}_a - \bar{X}_b|}{\sqrt{\frac{\sum X_{ia}^2 - m\bar{X}_a^2 + \sum X_{ib}^2 - n\bar{X}_b^2}{(m+n)-2}}}$$

where:

m = number of replication in one series
n = number of replications in the other series
$\bar{X}_a$ = mean of survivals per vial in one series
$\bar{X}_b$ = mean of survivals per vial in the other series
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The *t* values, together with the numbers of degrees of freedom (*df*) and the probabilities (*p*), are summarized for the experiments with *D. pseudoobscura* in table 5 and for *D. willistoni* and *D. paulistorum* in table 6. Examination of these tables leads to conclusions essentially simliar to those drawn from table 4: crosses between populations of *D. pseudoobscura* and *D. willistoni* tend to give a heterosis in the F_1 and a breakdown of the viability in the F_2, while in *D. paulistorum* the hybrids between populations do not usually differ in viability from their parents (except São Paulo × Içana). It may be noted that the only populations of *D. pseudoobscura* which failed to show either a heterosis in F_1 or a breakdown of the viability in the F_2 hybrids are those of Ferron, Utah, and of Gunnison, Colorado (see table 5). These populations are not only relatively close geographically (about 180 miles), but, and this may be more important, inhabit similar type of terrain.

Discussion

The genetic nature of differences between species and between races and local populations remains insufficiently understood. Some geneticists (e.g. Sturtevant, 1948) believe that species differ in substitution of relatively few gene alleles, the bulk of the genotype remaining rather constant during the evolutionary development. Other geneticists (Harland, 1936; Dobzhansky, 1941, 1951; Muller, 1942, 1950; and others) consider that the evolutionary process involves more extensive reconstructions of the genotype. The genotype of each species, and maybe of most races, is a system of interdependent genetic elements. A change in a part of such a system during race or species formation provokes reconstruction and re-integration of the whole system.

It is possible that the above points of view are both justified in different groups of organisms. Thus, it is tempting to speculate that the degree of integration of the genotype tends to be greater in the higher than in the lower forms. Genetic differences between strains of microorganisms may well be less extensive and less

TABLE 5. *The values of* t, *the numbers of degrees of freedom, and the probabilities for certain comparisons of the parental races of* Drosophila pseudoobscura *and their hybrids*

Localities	F_1			F_2		
	df	*t*	*p*	*df*	*t*	*p*
Gunnison—Gunnison × Mather	70	4.04	0.0001	104	2.12	0.02
Gunnison—Gunnison × Bryce	70	5.23	0.0001	106	1.75	0.07
Mather—Mather × Bryce	71	3.32	0.001	101	0.97	0.5
Mather—Gunnison × Mather	71	1.65	0.1	102	5.37	0.0001
Bryce—Mather × Bryce	72	2.68	0.01	103	0.94	0.3
Bryce—Gunnison × Bryce	72	2.21	0.03	106	2.71	0.01
Gunnison—Piñon × Gunnison	46	2.35	0.02	40	3.63	0.001
Piñon—Piñon × Gunnison	44	2.46	0.01	41	1.84	0.05
Bryce—Bryce × Piñon	46	0.36	0.7	38	2.10	0.05
Piñon—Bryce × Piñon	44	5.35	0.0001	41	0.84	0.5
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thoroughly interdependent, than those between races of species of higher plants, and especially of higher animals. Far too little is known at present about these matters to make considered judgment possible. The work described in the present article has been undertaken in an attempt to obtain some relevant evidence. The results show that even in organisms as closely related as the three species of *Drosophila* (*D. pseudoobscura, D. willistoni,* and *D. paulistorum*) the genetic situations may be different. In the two first named species the F_1 hybrids between populations living in different parts of the species area show heterosis, while the F_2 hybrids suffer a loss of the viability (hybrid breakdown). Among the three hybrids studied between populations of *D. paulistorum* only one showed indications of heterosis and none showed a breakdown.

The loss of the viability in the F_2 hybrids between geographically separate populations of the same species shows that the genotype of each of these populations represents a coherent system evolved under the control of natural selection. The gene recombination which occurs in the offspring of the hybrids between the populations results in formation of a multitude of genotypes, most of which are inferior in fitness not only to the F_1 hybrids but to the parental races themselves. It should be stressed that the data here presented do not warrant a conclusion that all the recombination genotypes are inferior in fitness. There exists good evidence to the contrary: Dobzhansky and Levene (1951) and Dobzhansky and Pavlovsky (1953) have shown that among the recombination products of race crossing in *D. pseudoobscura* there may occur some adaptively superior gene combinations. However, in at least a majority of the hybrids described in the present report, the gene recombination proved deleterious more often than beneficial.

The superior viability (heterosis) observed in some of the F_1 hybrids between populations is more difficult to account for. It should be emphasized that the geographic populations hybridized in our experiments can not be regarded as inbred. The experimental populations were hybrids between eight (in *D. pseudoobscura*), or between ten (in *D. willistoni* and *D. paulistorum*) strains from each locality (see above). Populations so created must have had a supply of genetic variance comparable to the natural populations which they represented. And yet, the F_1 hybrids between these populations showed an increased fitness, at least under experimental environments. The result obtained may seem paradoxical: experi-

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TABLE 6. *The values of* t, *the numbers of degrees of freedom and the probabilities for certain comparisons of the parental races of* Drosophila willistoni *and* Drosophila paulistorum *and their hybrids*

Localities	*Drosophila willistoni*						*Drosophila paulistorum*											
							First experiment						Second experiment					
	F_1			F_2			F_1			F_2			F_1			F_2		
	df	*t*	*p*	*df*	*t*	*p*	*df*	*t*	*p*	*df*	*t*	*p*	*df*	*t*	*p*	*df*	*t*	*p*
Belem—Belem × Içana	36	0.81	0.4	36	1.74	0.1	33	0.37	0.6	35	1.10	0.2	36	0.22	0.7	44	0.54	0.5
Belem—Belem × São Paulo	36	2.27	0.02	35	0.23	0.7	32	1.00	0.3	34	1.11	0.2	36	1.07	0.3	44	0.11	0.7
Içana—Belem × Içana	35	0.14	0.8	36	3.33	0.0001	35	1.04	0.3	36	0.19	0.7	36	0.14	0.7	44	0.42	0.6
Içana—São Paulo × Içana	35	2.10	0.05	36	0.39	0.7	33	2.12	0.05	34	1.11	0.2	36	2.15	0.05	44	1.39	0.2
São Paulo—Belem × São Paulo	35	2.10	0.05	35	0.99	0.3	33	0.85	0.5	35	2.08	0.05	36	0.00	0.00	44	0.32	0.6
São Paulo—Içana × São Paulo	35	2.93	0.01	36	0.71	0.5	32	3.44	0.0001	34	0.46	0.5	36	0.85	0.3	44	0.86	0.3

mental populations have fitness greater than natural populations.

The origin of this paradoxical situation is for the time being a matter of speculation. A question which arises in this connection is this: are the F_1 hybrids superior to their parents only in the competitive ability of their larvae under conditions of crowding and insufficient food, or will they show superiority also in other respects, such as fecundity, longevity, etc.? Experiments which should produce data that may help to answer the above questions are now under way. If the F_1 hybrids are really heterotic, it will be necessary to suppose that heterozygosis per se is, up to a certain point, a viability stimulant. Such a hypothesis, favored by some of the early students of genetics of heterosis, is indeed suggested not only by the data presented in this paper, but also by those of Brncic (1954), and especially by those of Lerner (1954). Further discussion of this problem may well be postponed until more data are available. It may, however, be pointed out that the fact that the heterosis observed in the F_1 hybrids between populations is a phenomenon which is observed under artificial laboratory conditions, and is presumably not exploited in nature, is, after all, not as difficult to understand as it may seem at the first glance. Indeed, this heterosis is ephemeral, being restricted to the F_1 generation of hybrids, and reversed in the F_2 generation, in which the favorable effects of heterozygosis are offset by the hybrid breakdown. Maintenance of such a heterosis in natural populations is, then, difficult in a sexually reproducing species.

SUMMARY

The viability under controlled laboratory conditions was studied in 5 geographically distinct populations of *Drosophila pseudoobscura* and their F_1 and F_2 hybrids. A similar comparison was made of 3 geographic populations of *D. willistoni* and their hybrids, and of an equal number of populations and of hybrids of

D. paulistorum. The technique consisted in placing 10 or 20 freshly hatched larvae from the population to be studied in a vial with a measured small amount of food, together with 10 or 20 fresh larvae of an easily recognizable mutant type. The numbers of adult flies which developed from these larvae were determined and recorded.

The results show (tables 1–6 and figures 1 and 2) that, in *D. pseudoobscura* and *D. willistoni*, the F_1 hybrids between geographically different populations are superior in viability to the parental populations. Such heterosis has been observed in only one of the three crosses made in *D. paulistorum*. The F_2 hybrids between the geographic populations not only loose the heterosis present in the F_1, but are in fact usually inferior to the parental populations. Such a hybrid breakdown is much more pronounced in *D. pseudoobscura* and *D. willistoni* than in *D. paulistorum*. In the last named species, only one of the three crosses showed an indication of a loss of vigor in the F_2 generation.

It is concluded that the genotoypes of geographic populations are integrated systems of genetic elements, which have arisen in the evolutionary process under the control of natural selection. Gene recombination in the offspring of hybrids between such populations leads in some species to formation of numerous genotypes most of which (though not all) have a lowered fitness. The heterosis observed in the F_1 hybrids between geographic populations may be due to the heterozygosis for many gene loci acting as a viability stimulus. These conclusions are subject to validation or disproof by further experiments, which are in progress.

Acknowledgments

I wish to acknowledge my obligations to Professor Th. Dobzhansky for his interest and for his kindness and advice. I am also grateful to R. C. Levontin for his help with statistical methods. I wish to express my gratitude to the East European Fund, which provided a partial support of this work.

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HYBRIDIZATION IN THE RED-EYED TOWHEES OF MEXICO

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Received February 15, 1954

INTRODUCTION

The present interest of students of evolution in the subject of hybridization is due in part to the relatively recent discovery that fertile back-crossing interspecific hybrids greatly increase the variability in both parental species. The resulting expansion of the genetic spectrum provides a larger range of variants upon which selection can operate and may therefore be an important evolutionary factor.

The possible role of hybridization in evolution was pointed out by Lotsy in 1916 but his arguments lost much of their value because he insisted that hybridization alone was the basis of evolution. Cockayne and Allan (e.g., 1926, 1927) in several papers described hybrid plants from New Zealand and discussed the possible role of hybridization in evolution. In 1928 Ostenfeld summarized the literature to that date and further emphasized the importance of hybridization in the production of new recombination types, especially in cultivated plants. In 1930 the English ornithologist P. R. Lowe, who had been impressed by the observations of Lotsy and Cockayne, suggested a hybrid origin for certain avian species.

During a decade (1928–1938) of study of wild and cultivated hybrids in such plant genera as *Iris* and *Tradescantia*, Edgar Anderson developed a set of techniques for the detection and evaluation of hybridization. In 1938 Anderson and Hubricht proposed the term "introgressive hybridization" " to denote the gradual infiltration of the germplasm of one species into that of another as a consequence of hybridization and repeated backcrossing" (Anderson, 1953: 282).

Introgression is, of course, only an expanded kind of recombination but the greatly increased genetic latitude which results from gene exchange between differently adapted populations provides an enormously greater variability than can result from intraspecific recombinations wherein variation has been reduced by selection to a single adaptive peak for the population.

Introgressive hybridization was observed by Clausen in Danish populations of *Viola* as far back as 1921 and 1922 (Clausen, 1951: 16). The studies of Riley (1938, 1939) on *Iris* and the many papers by Anderson and his students have been summarized by Anderson (1949, 1953).

The full significance of hybridization as a factor in evolution is still under debate. In 1949 (p. 102) Anderson felt unable to assess its importance while pointing out that in some instances it might exceed mutation as a source of new genetic material. By 1953 Anderson had overcome his reluctance to take a firm stand on the question and clearly stated his belief that, "introgressive hybridization is certainly more important than all other factors combined in providing raw material for natural selection to work upon" (1953: 300). It is Anderson's contention that in plants introgression is a major cause of variability and that it far exceeds mutation in this regard. His enthusiasm is not completely shared by Stebbins (1950: 279) who states that, "Introgressive hybridization, whatever may be its importance in modifying and amplifying the variation pattern of certain individual species, is nevertheless by its very nature not a way of producing new morphological or

physiological characteristics, and therefore of progressive evolution. It merely produces convergence between previously more distinct species." Stebbins partly contradicts this statement in the next sentence which reads, "There is, however, evidence that in some instances hybridization can result in the appearance of types which are actually new." These, he indicates, involve the establishment of subspecies which arise from hybridization between preexisting subspecies of the same species, provided a new and suitable habitat is available to them.

Evidence that introgressive hybridization can produce morphological characteristics in the hybrids which transgress the variation of the parental species has been obtained by Smith (1954, in press). Results of experimental crosses between *Nicotiana langsdorfii* and *N. sanderae* are described by him as follows, "All the selected lines and backcrosses are within the parental range of corolla size except that the cross of the large diploid F_5 selection with *N. sanderae* exceeds the large parent. This result with a polygenic character, together with the appearance of new corolla colors by recombination of simple Mendelian genes, demonstrates transgression of parental characteristics by introgressive hybridization."

Grant (1953) presents evidence for the hypothesis that the Angiosperms have evolved as a succession of hybrid complexes resulting in the present reticulate pattern characterized by numerous taxonomically "difficult" groups. This is in contrast to the situation in most animal groups where divergent lines of evolution seem to be the rule.

Dobzhansky (1951: 297–300) briefly reviews the literature but does not take a stand regarding the relative importance of introgression versus mutation. He endorses Stebbins' (1950: 252–254) explanation of the greater frequency of hybridization which is found in plants as compared with animals. Stebbins points out that because the individual life span of plants which reproduce vegetatively may be of the order of hundreds or thousands of years, the maintenance and spread of the population is guaranteed by asexual means and sexual reproduction, by seeds, serves mainly to maintain the evolutionary plasticity. The production of ill-adapted hybrids will not be as severely selected against in this system as in sexually reproducing animals where the species is entirely dependent upon cross-fertilization. This argument also provides an explanation for the fact that isolating mechanisms in animals are less subject to breakdown than those in plants.

Nevertheless, introgressive hybridization must be considered as adequately demonstrated in animals (Meise, 1936; Sweadner, 1937; Blair, 1941; Chapin, 1948; Hovanitz, 1949; Sibley, 1950).

Interspecific avian hybrids have been known and their origins understood for well over a century. Several hundred hybrid birds have been described. It is within the past 20 years however, that most, if not all, of the significant analytical papers on avian hybrids have appeared. In a bibliography of nearly 800 titles concerning hybridization in birds I find only 20 which may be called "analytical." All of these have appeared since 1930. Prior to that time most papers concerned with hybrids were merely notices of their occurrence.

The present paper presents the results of a continuation of previous studies on hybridization and associated introgression in the red-eyed towhees of the avian genus *Piplio* in Mexico. Frequent reference must be made to the previous paper (Sibley, 1950) but repetition of data will be avoided whenever possible.

Materials

The present study is based upon 351 specimens from the states of Jalisco, Michoacan, Nayarit, Puebla and Vera Cruz collected between July 8 and September 11, 1950. These specimens were given to the Museum of Vertebrate Zoology of the University of California. The previous paper (*op. cit.*) utilized 779

specimens from Mexico, thus providing an actual basis of 1130 specimens from Mexico at the present time. The majority of these show evidence of hybridization.

The Parental Species

Hybridization implies that two unlike parents have interbred. (See Mayr, 1942: 258; 1950: 102 or Sibley, 1950: 155, for the application and definition of the term "hybrid"). In this instance the parental species involved are the Spotted (Red-eyed) Towhee (*Pipilo erythrophthalmus*), and the Collared Towhee (*Pipilo ocai*). Detailed descriptions (p. 111) and a colored plate (pl. 11) are provided in the 1950 paper. Ridgway (1901: 406 and 410) also contains descriptions of these species.

The Analysis of Hybrids

When the previous study was in progress (1946–1948) a method for the analysis of hybrids was devised. This system, which was termed a "hybrid index" (1950: 111–113) proved most useful in the analysis and demonstration of the character gradients due to introgressive hybridization. It was not until the 1950 paper was in press that I obtained a copy of Anderson's interesting little book (1949) and discovered that "my" method had been anticipated to the last detail by at least 12 years! In spite of Anderson's indictment of this method as "crude" (1949: 88) it proves to be extremely useful in the analysis of color gradients due to hybridization.

The "pure" parental species, *P. erythrophthalmus* and *P. ocai* differ in plumage color in six principal areas (1950: 112) as follows:

1. Pileum color: Chestnut in *ocai*, black in *erythrophthalmus*.
2. Back and wing spots: White spots on scapulars and wing coverts in *erythrophthalmus*. These areas are unspotted in *ocai*.
3. Back color: Green in *ocai*, black in *erythrophthalmus*.
4. Throat color: White in *ocai*, black in *erythrophthalmus*.
5. Flank color: Brownish olive in *ocai*, rufous in *erythrophthalmus*.
6. Tailspots: White spots on three outer rectrices in *erythrophthalmus*, absent in *ocai*.

It was found that for each of the six characters five gradations could be distinguished. These five gradations were indicated by the numbers, 0, 1, 2, 3, 4.

Any character expressed as fully as in pure *ocai* was scored "0", one expressed as fully as in pure *erythrophthalmus* was scored "4". Exact intermediates were scored "2" and those between the midpoint and one of the pure parents were scored either "1" or "3". Since there were six characters, each with a possible variation in the hybrids from "0" to "4" a pure *ocai* would have a summated score of 0 ($6 \times 0 = 0$) and a pure *erythrophthalmus* would score 24 ($6 \times 4 = 24$). The summation of the characters of a hybrid specimen will fall on the 0–24 scale between the pure parents.

Figure 1 indicates the average hybrid index numbers for the populations which have been sampled.

Measurements of Size

The weights of 325 of the 351 specimens collected in 1950 were obtained. Linear measurements from skins, in millimeters, were taken as follows: wing, the chord; tail, from insertion of the two middle rectrices; tarsus, from the joint between the tarsus and the tibia (the heel) to the midpoint of the distal margin of the most distal undivided scute; bill, from anterior margin of the nostril to the tip of the maxilla.

Summary of Previous Results

The map (figure 1) indicates the distributional pattern of the red-eyed towhees of Mexico as presently understood. *Pipilo erythrophthalmus* occurs primarily in the oaks and brushy undergrowth in the Sierra Madre Occidental, the Sierra Madre Oriental, and south in the mountains bordering the eastern edge of the plateau to Oaxaca. A race (*chiapensis*)

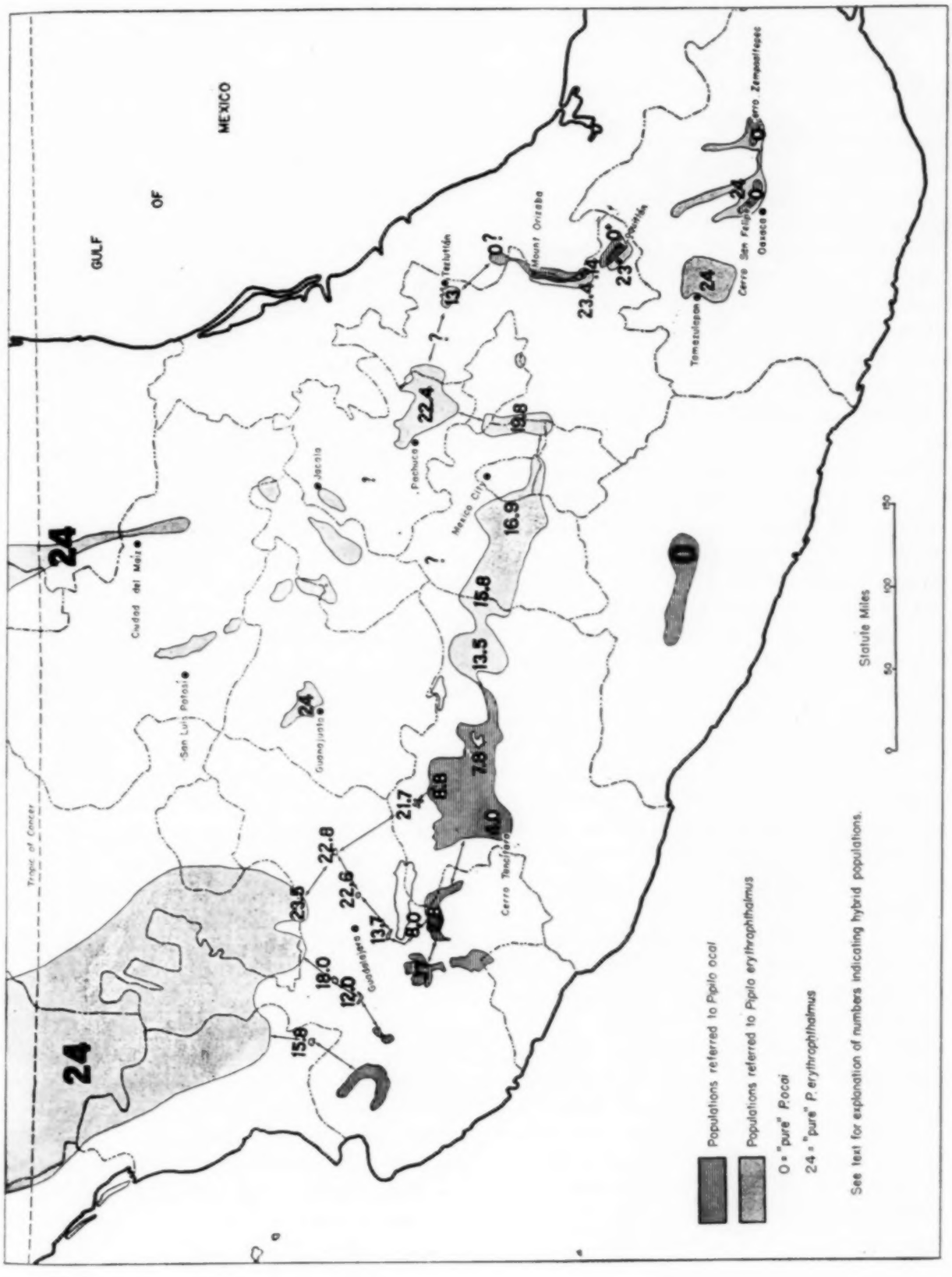

FIG. 1. Distribution of the Red-eyed Towhees in central Mexico.

occurs in the highlands of Chiapas and Guatemala.

Pipilo ocai occurs primarily in coniferous woodland and associated undergrowth in the Sierra Madre del Sur in Guerrero, the highlands of central Oaxaca, the high country centering at Mount Orizaba, and the mountains of western Jalisco.

In Oaxaca (Cerro San Felipe) the two species have been found in a condition of complete sympatry with no evidence of interbreeding (1950: 152–155). On the west slope of Mount Orizaba, Puebla, where the two species are again in contact, approximately 16% of the 117 known specimens from the area of sympatry show evidence of hybridization (1950: 155–165).

The series of populations which extend east and west across the plateau from the vicinity of Mexico City (Mount Popocatepetl) to Jalisco have been called the "transplateau gradient" (1950: 169–176) and represent a partially stabilized hybrid cline which owes its existence to introgressive hybridization between the parental species; *ocai* in Jalisco at the west end, and *erythrophthalmus* in Hidalgo at the east end. As will be shown, there are several other introgressive gradients operating between the Jalisco and Michoacan populations and those to the northwest in the Sierra Madre Occidental (*P. e. griseipygius*).

On the summit of Cerro Viejo, Jalisco, there occurs a hybrid population which is partially isolated from other populations. Seventy-six specimens from Cerro Viejo were analyzed (1950: 165–169) by the hybrid index method. No two were exactly alike and the range of variability showed a spread of 15 index units, from "6" to "20". The Cerro Viejo population is thus 15 times more variable in plumage color than either of the pure parents. The average hybrid index for the 76 specimens from Cerro Viejo is "13.7".

Nomenclatural Problems

Because of the sympatric situation in Oaxaca and the pattern of variability in the hybrid populations it was decided that the best taxonomic practice was to consider *ocai* and *erythrophthalmus* to be different species. That *ocai* and *erythrophthalmus* are not fully formed species is apparent but that they are *not subspecies* is equally apparent. The term "semispecies," although without categorical rank, seems to describe the situation.

The 1950 Field Study

From the summit of Cerro Viejo in 1948 it was possible to see several mountain tops which seemed to support a cover of oaks and brush and which were deemed likely sites for other mountain-top hybrid populations. Time did not permit further exploration in 1948 but all such mountains were noted for future reference. When Mr. J. R. Alcorn prepared to visit Cerro Tequila in western Jalisco in 1949, in the interests of the University of Kansas, he was urged to collect a sample of the towhee population. The single adult and three juvenal specimens which he obtained on Cerro Tequila (Sibley, 1950: 169) were undeniable hybrids. This discovery provided the proof of previous suspicions and plans were laid for field work in 1950, the primary purpose being to ascend and obtain samples of the towhee populations from as many of the isolated mountains in southwestern Mexico as possible (see figure 2).

A total of 12 collecting localities yielded specimens of towhees in 1950. Seven of these were mountain tops located in Jalisco, Nayarit, and Michoacan; two were additional localities in the "transplateau gradient"; one was at the southern tip of the Sierra Madre Occidental, and two were at the eastern edge of the plateau in Puebla and Vera Cruz. These 12 localities, the dates visited, and the number of towhee specimens (in parentheses) from each are as follows.

1. Cerro Tequila, 6,600′, 7 mi. S. Tequila, Jalisco. July 8–15. (69)
2. Sierra de Ameca, 7,000′, 6 mi. N. Ameca, Jalisco. July 19–24. (18)

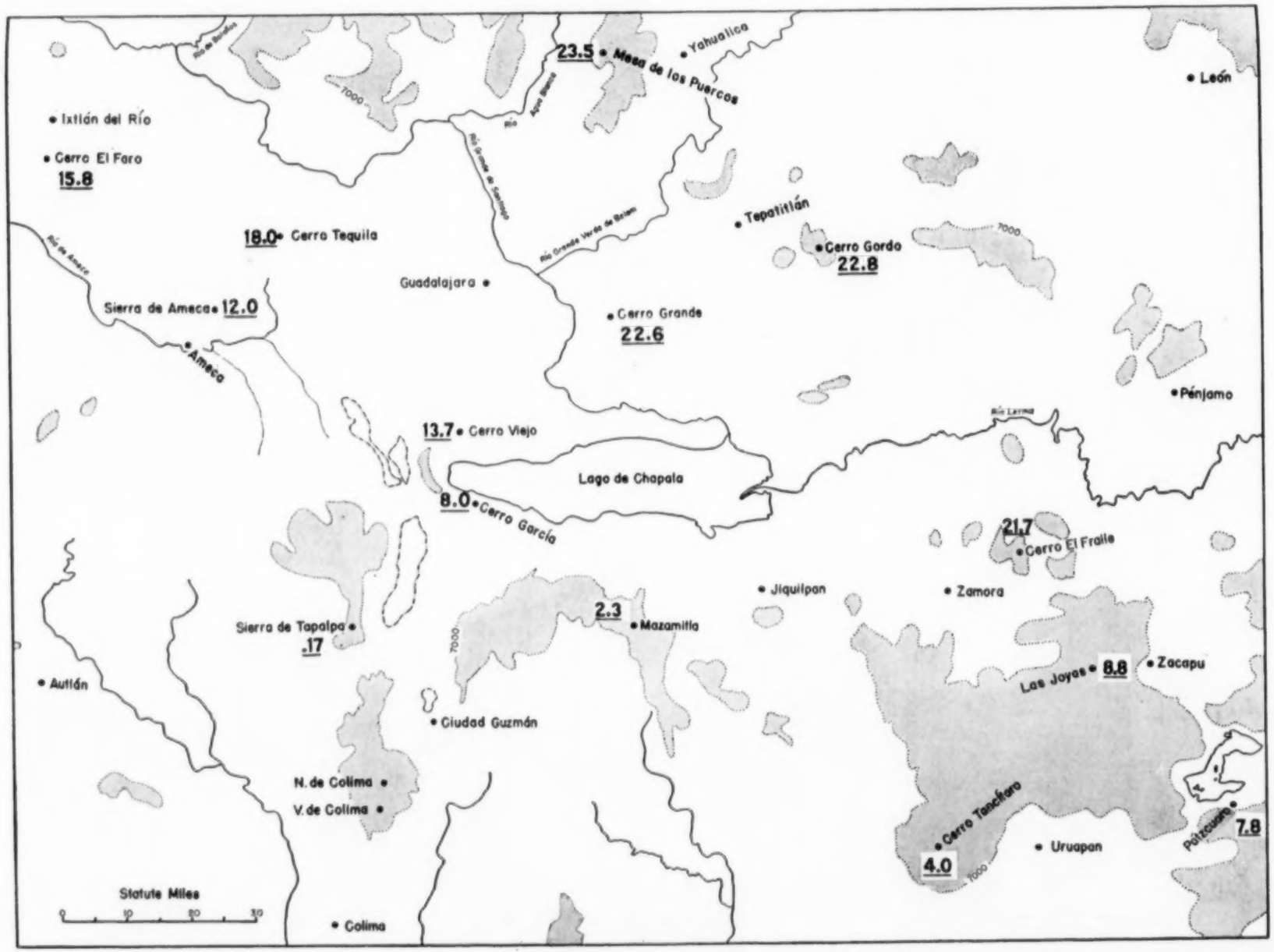

FIG. 2. Dark areas indicate presumably favorable towhee habitat above 7,000 feet altitude in southwestern Mexico. Numbers are average hybrid indices for population samples.

3. Cerro García, 8,500′, 32 mi. S. Guadalajara, Jalisco. July 30–Aug. 3. (58)
4. 4 mi. W. Mazamitla, 6,800′, Jalisco. Aug. 5–7. (29)
5. Cerro Gordo, 8,000′, 13 mi. ESE Tepatitlán, Jalisco. Aug. 8–10. (42)
6. La Mesa de los Puercos, 8,000′, 12 mi. W. Yahualica, Jalisco. Aug. 11–12. (14)
7. Cerro Grande, 7,600′, 18 mi. ESE Guadalajara, Jalisco. Aug. 14–17. (36)
8. Cerro El Fraile, 8,200′, 14 mi. NE Zamora, Michoacan. Aug. 22–24. (16)
9. Las Joyas, 7,700′, 12 mi. W. Zacapu, Michoacan. Aug. 24–26. (12)
10. Cerro El Faro, 6,800′, 6 mi. S. Ixtlán del Río, Nayarit. Aug. 29–Sept. 2. (31) (This mountain is also known as "Llorón de Ixtlán.")
11. 3 mi. W. Teziutlán, 7,300′, Puebla. Sept. 7–9. (11)
12. Puerto Morelos, 8,000′, 19 mi. WSW Orizaba, Vera Cruz. Sept. 10–11. (11) *P. erythrophthalmus;* (4) *P. ocai.*

Previously taken specimens were reported upon in the 1950 paper. These other localities and numbers of specimens from southwestern Mexico are as follows:

1. La Laguna (6,500′, 10 mi. NE Mascota) Jalisco. (10) U. S. Biol. Surv., U. S. Nat. Mus. and Amer. Mus. Nat. Hist.
2. Los Masos, southern Jalisco. (4) Amer. Mus. Nat. Hist.
3. Sierra Nevada (= Nevado de Colima), Jalisco. (1) U. S. Biol. Surv.
4. La Pisagua, Jalisco. (2) Amer. Mus. Nat. Hist.
5. La Cumbre (near Mascota), Jalisco. (1) A.M.N.H.
6. Sierra de Tapalpa, 7,450′, 5 mi. W. Sayula, Jalisco. (9) M.V.Z.
7. Lake Pátzcuaro and vicinity. (36) M.V.Z., G. M. Sutton coll., U. S. Biol. Surv.
8. Mt. Tancitaro and vicinity. (20) U.S.B.S., Chicago N. H. Mus.
9. Cerro Viejo, 9,000′, 22 mi. S. Guadalajara, Jalisco. (77) M.V.Z. and A.M.N.H.

The Distribution of Red-Eyed Towhees in Relation to the Topography and Vegetation in Southwestern Mexico

In order to understand and interpret the causes and results of hybrization between *Pipilo erythropthalmus* and *Pipilo ocai* it is imperative to understand the habitat requirements of each species and the complex topography of the areas where the hybrid populations occur.

As previously pointed out, *Pipilo erythrophthalmus* is principally adapted to live in the understory vegetation associated with the oak or pine-oak woodland in southwestern Mexico. *Pipilo ocai* is restricted to the understory of the pine-oak or fir forests, usually at higher elevations than *P. erythrophthalmus.* In southwestern Mexico neither species, nor the hybrid populations resulting from their interbreeding, occur below 5,500 feet during the nesting season, and most breeding populations are found above 7,000 feet. Thus, any area in southwestern Mexico which lies below 5,000 feet or which lacks suitable vegetative cover will function as a barrier. Lowland areas of unsuitable vegetative environment such as river valleys and open plains are the primary barriers to these sedentary birds. That all such barriers are only partial is indicated by the existence of the isolated mountain top hybrid populations.

The following description of topography and vegetation is based primarily on my own observations and field notes. These have been supplemented by reference to the paper by Leopold (1950), the book by Goldman (1951) and the World Aeronautical Charts (Nos. 590, 642, 643) published by the U. S. Coast and Geodetic Survey. Reference to the maps (figures 1, 2, 3, 4) will help to clarify the following description.

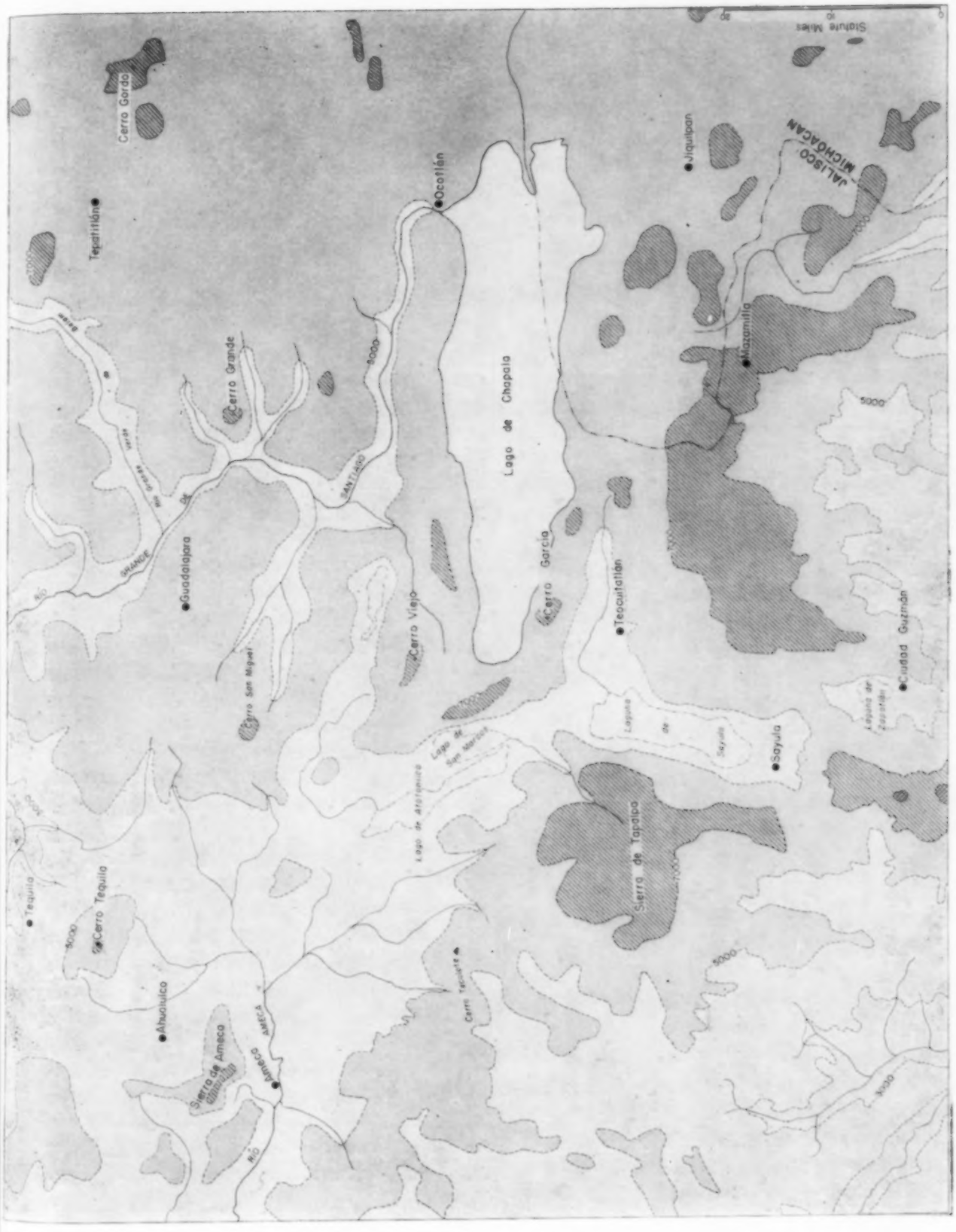

FIG. 3. Parts of Jalisco and Michoacan indicating certain localities and their topographic relationships.

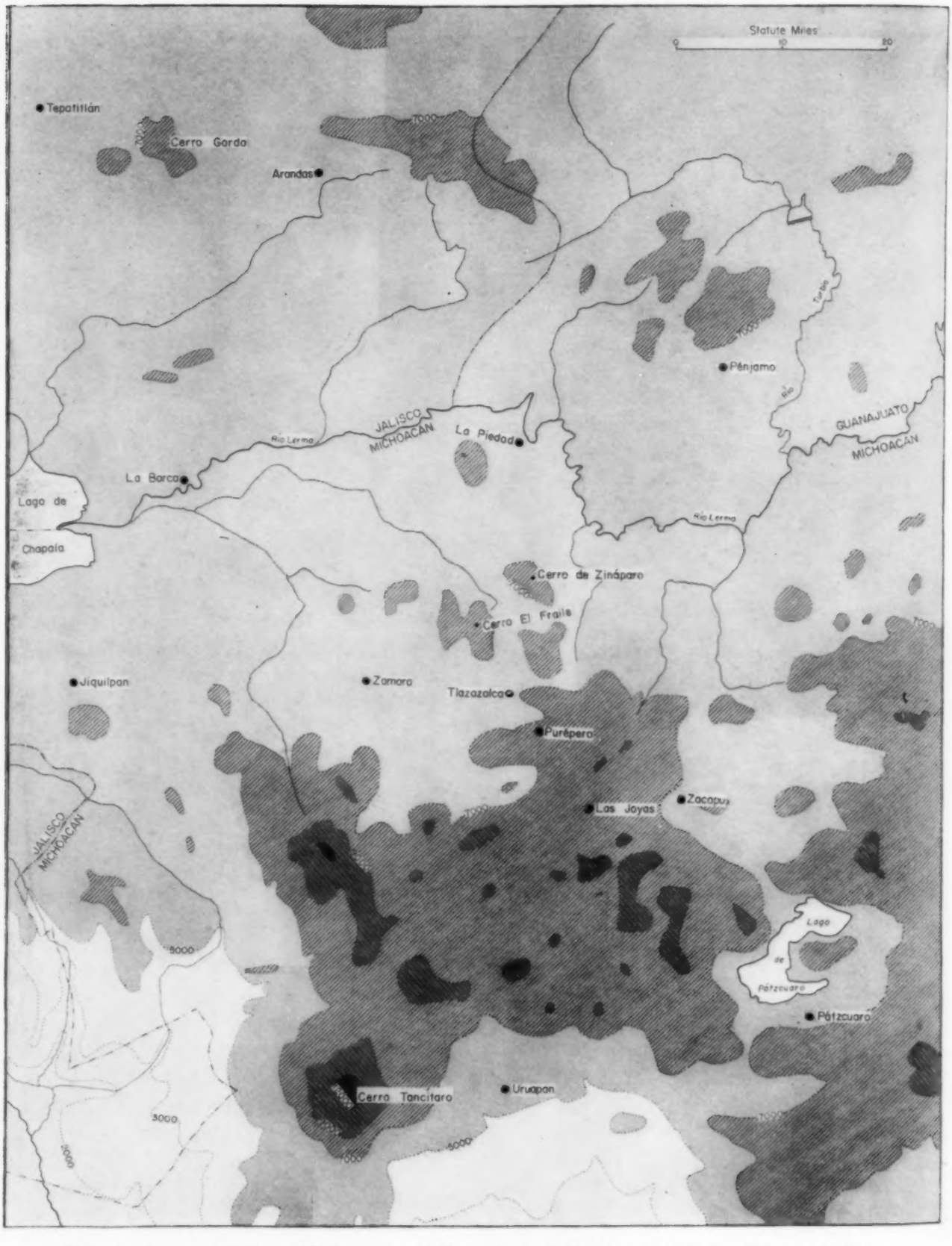

FIG. 4. Parts of Jalisco, Michoacan and Guanajuato indicating certain localities and their topographic relationships.

The base level of the plateau in southwestern Mexico slopes gradually from the north and east toward the west and south. León is at an elevation of 5,900 feet, Guadalajara is at 5,200 feet. To the west of Guadalajara, as the edge of the plateau is approached, the country is highly dissected by streams and broken by numerous mountains and small ranges. The plateau between Guadalajara and León is covered with a mesquite-grassland association. Much of the area is used as pasture or is under cultivation. This area is approximately bounded by the Río Grande Verde de Belem on the northwest, the Río Grande de Santiago to the southwest and the Río Lerma on the south. Rising from this relatively flat plain are many isolated hills and small ranges, for example, Cerro Grande and Cerro Gordo, which support oaks and brush and which harbor breeding populations of towhees.

The Río Grande de Santiago and its tributaries form the principal drainage system of the southwestern plateau. The Santiago is below 5,000 feet throughout its course, and below 4,000 feet for most of it. It is therefore an important barrier to towhees. North of the Río Santiago are the southernmost portions of the Sierra Madre Occidental where *Pipilo erythrophthalmus griseipygius* is resident above 7,000 feet.

The Río Lerma has its origin far to the east in the Lerma marshes near Toluca and flows westward to Lake Chapala at 5,000 feet elevation. The Río Lerma is probably less effective as a barrier than the Río Santiago for it is above 5,000 feet throughout its course and does not occupy a deep gorge like that of the Santiago.

The Río Ameca has its headwaters a few miles to the west of Lake Chapala. A range of low hills lies between. The lowlands associated with the Ameca drainage are below 5,000 feet and are continuous with the playa lakes of Atotonilco, San Marcos, Sayula and Zapotlán. This lowland area continues southward beyond Ciudad Guzmán and connects with the lowlands of the Río Naranjo. Thus there is a continuous lowland barrier to towhees which separates the Sierra de Tapalpa, the Nevado de Colima highlands, and the mountains south of the Río Ameca from the highlands to the north and east. West and south of this lowland barrier in the Sierra de Tapalpa, Nevado de Colima, etc. occurs *Pipilo ocai alticola*.

Between the Río Ameca and the Río Santiago there rise a number of isolated volcanic peaks and small ranges. The base level of the plateau averages 4,000 feet and the plant cover of this lowland area is primarily xerophytic, with cactus, mesquite and grassland. Above 6,000 feet the mountains support oak or pine-oak woodland with an understory vegetation suitable for towhees. On several of these peaks (Cerro Tequila, Sierra de Ameca, Cerro El Faro, Cerro Viejo) occur hybrid populations of towhees. The unfavorable lowlands and the river systems are barriers to free panmictic movements.

South of Lake Chapala is a large highland, the Sierra del Tigre, in which Mazamitla is situated, and which is isolated from the Sierra de Tapalpa by the low country between. South of the Sierra del Tigre highlands the descent is rapid toward the coastal lowlands. The extensive highlands to the east include the Cerro de Tancítaro (12,660 feet) and several other peaks of more than 10,000 feet in elevation. There is no important lowland barrier between Mazamitla and the Pátzcuaro area. All of these mountain regions support forests of oaks, pines and firs. The towhee populations which occur at Mazamitla, Cerro Tancítaro, Las Joyas and Pátzcuaro are parts of the trans-plateau hybrid gradient. The name *Pipilo ocai negrescens* has been applied to the population of the Pátzcuaro region.

Cerro García, at the southwestern corner of Lake Chapala, is an abruptly rising volcanic peak which is surrounded by lowlands supporting a xerophytic cover unfavorable for towhees. The hybrid population of towhees occurs above

7,000 feet in the understory of the dominant oak-madrone woodland.

Cerro El Fraile is one of a group of isolated peaks supporting an oak-madrone woodland on the summit. The pines do not extend northward to El Fraile from the vicinity of Las Joyas.

The photographs, figures 12–19, illustrate the topographical and vegetational relationships of some of these localities.

The Hybrid Populations

In describing and analyzing the hybrid populations it is appropriate first to describe the parental species and their geographic relationships to the hybrids.

The Pipilo erythrophthalmus component

North of the Río Grande de Santiago, in the Sierra Madre Occidental, the resident towhee is *Pipilo erythrophthalmus griseipygius*. At La Mesa de los Puercos, 12 miles west of Yahualica, Jalisco, the resident birds are very close to "pure" *P. e. griseipygius*. The hybrid index is "23.5" for the 13 specimens available. All individuals exhibit fully developed (= "4") *erythrophthalmus* characters for back spotting, back color, flank color, and tail spots. Four show chestnut shaft streaks in the crown (= "3") and three have a small amount of visible white in the throat (= "3"). These are the only readily detectable indications of introgression in this sample. See table 1 for the measurements of these specimens. A sample of 11 adults from localities in Durango, Sinaloa, Zacatecas and Nayarit (1950: 139–140) did not yield evidence of introgression.

The birds at La Mesa de los Puercos are separated from the main ranges of the Sierra Madre Occidental by the deep gorge of the Río Agua Blanca and lowlands to the north. The barrier toward the southeast, across the Río Grande Verde de Belem, is also large but may be less complete. See figure 12.

At La Mesa de los Puercos the towhee population was just past breeding, mostly silent and beginning to molt on August 11–12, 1950. On Cerro Gordo, only 44 miles (air line) distant, where towhees were collected during the preceding two days (Aug. 8–10) the birds had been in full song and not yet far into the molt.

The vegetation at La Mesa de los Puercos is dominantly pine, oak and madrone with extensive areas of manzanita brush and a few patches of *Baccharis* on exposed slopes. The towhees were found mostly in the manzanita thickets. The general aspect of the country indicated a lower rainfall than in the Cerro Gordo region.

It may safely be assumed that the population at La Mesa de los Puercos is one of those contributing to the introgressive hybrid clines which extend to the south.

Table 1. *Measurements of La Mesa de los Puercos population*

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight	♂	10	42.65 ± 0.62	1.97	4.62
	♀	3	42.46 ± 2.32	4.02	9.47
Wing	♂	10	86.72 ± 0.65	2.06	2.37
	♀	3	82.83 ± 1.58	2.74	3.31
Tail	♂	10	100.06 ± 1.14	3.60	3.59
	♀	3	95.83 ± 1.66	2.87	2.99
Tarsus	♂	9	27.95 ± 0.28	.83	2.96
	♀	3	27.83 ± 0.17	.29	1.04
Bill from nostril	♂	10	9.70 ± 0.12	.39	4.00
	♀	3	9.57 ± 0.15	.25	2.66

The Pipilo ocai component

The mountains south of the Río Ameca drainage and west of the Sayula-Ciudad Guzmán lowlands are inhabited by a race of the Collared Towhee, *P. ocai alticola*. In the Sierra de Tapalpa this population inhabits a relatively humid, open pine-oak woodland above 6,500 feet. The birds occur in thickets of acacia, hawthorne and *Baccharis*. A series of 29 adults was available for study (1950: 149). The hybrid index for these birds was ".17" which indicates a slight amount of introgression from *erythrophthalmus*.

The effects of introgression are indicated primarily by the lack of a median frontal white line, reduced white superciliary line and the occasional presence of white tips on the outer rectrices. The other races of the Collared Towhee, *P. o. ocai* of the eastern edge of the plateau (Mount Orizaba, etc.), *P. o. brunnescens* of Oaxaca, and *P. o. guerrerensis* of the Sierra Madre del Sur in Guerrero, all have well-marked white frontal lines and superciliary stripes. The reduction of these markings in *P. o. alticola* is apparently the result of increased melanins in the head plumage due to introgression from *P. erythrophthalmus*. Introgression will be shown to come from at least two directions, namely, from the east via the transplateau gradient and from the north via the hybrid populations of Cerro Tequila, Sierra de Ameca, Cerro El Faro, etc.

In summary: So far as is known at this time the source of *Pipilo erythrophthalmus* genes is the population of *P. e. griseipygius* which is resident north of the Río Santiago and the Río Grande Verde de Belem. The source of *Pipilo ocai* genes is the population of *P. o. alticola* which occurs in the mountains south of the Río Ameca and west of Sayula and Ciudad Guzmán. Both of these populations are themselves "tainted" by introgression but represent the closest approaches to "pure" populations of each of the parental species in the area under immediate consideration. Phenotypically "pure" *P. erythrophthalmus* occurs to the north in the Sierra Madre Occidental and "pure" *P. ocai* occurs in the mountains of Guerrero. (It is apparent that there is no certainty that introgression does not also affect these latter populations, hence the word "pure" in quotes.)

The Introgressive Gradients

The first introgressive cline which will be analyzed is that which includes the populations of La Mesa de los Puercos, Cerro Gordo, Cerro Grande, Cerro Viejo, Cerro García, and Mazamitla. The map, figure 2, indicates the geographic relationships of these localities.

Cerro Gordo

The base level of the plateau at Tepatitlán, 13 mi. WNW of Cerro Gordo, is 5,400 feet. The rise is gradual to the base of the mountain. There are good stands of oaks at 6,000 feet and a single towhee was heard at 6,500 feet, nearly three miles from the beginning of the last steep rise up Cerro Gordo. The density of the towhee population increased sharply above 7,200 feet to the summit at 8,700 feet.

On Cerro Gordo the vegetation was ideal for towhees; dense shrubby ground cover, including *Baccharis* and other shrubs, in an oak-madrone woodland. No pines were found.

The topography and vegetation at Cerro Gordo are illustrated by figure 13.

The 42 specimens from Cerro Gordo range from "22" to "24" with an average hybrid index of "22.8". The effects of introgression are primarily indicated by frequent chestnut shaft streaks in the crown (28 specimens are rated "3" for crown color), and visible white in the throat (24 specimens are rated "3" for throat color). In the other four characters the Cerro Gordo birds show no detectable *P. ocai* influence in the plumage coloration. See table 2 for the measurements of this population.

From La Mesa de los Puercos to Cerro Gordo is 44 miles air line distance. In spite of the barrier of lowlands and the

TABLE 2. *Measurements of Cerro Gordo population*

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight	♂	28	42.56 ± 0.46	2.44	5.73
	♀	13	40.11 ± 0.61	2.21	5.50
Wing	♂	28	88.29 ± 0.37	1.99	2.25
	♀	14	82.88 ± 0.57	2.13	2.56
Tail	♂	28	102.15 ± 0.58	3.06	2.99
	♀	13	95.61 ± 0.83	2.99	3.12
Tarsus	♂	27	27.74 ± 0.14	.74	2.65
	♀	14	27.13 ± 0.28	1.03	3.79
Bill from nostril	♂	28	9.32 ± 0.09	.49	5.27
	♀	14	9.28 ± 0.13	.48	5.15

Río Grande Verde de Belem the shift in hybrid index is extremely small, being only .7 of an index unit or .016 index unit per mile.

Cerro Grande

Thirty-five miles southwest of Cerro Gordo is this mountain which rises from 4,800 feet at the base to 7,800 feet at the summit. It is situated at the edge of the Río Santiago lowlands which drop to approximately 4,500 feet at the river itself. On the north side below 6,000 feet, the vegetation is dominantly thorn scrub. At 6,300 feet the first oaks were encountered and at 6,800 feet the first towhees. From 7,000 feet to the summit they were common in dense thorn scrub undercover mixed with the oak woodland. Here, as an Cerro Gordo, towhees were the most abundant species of bird.

The average hybrid index, based on 36 specimens, is "22.6", ranging from "17" to "24". The effects of introgression from *ocai* are again primarily detectable in the pileum and throat but there is in addition a noticeable reduction in the back spotting in seven specimens. The pileum color is "3" in 15 specimens, "2" in one and "1" in one. The remaining 19 have solidly black crowns ("4") as in pure *erythrophthalmus*. See table 3 for data on measurements.

Between Cerro Gordo and Cerro Grande there are numerous hills which support brush and oaks. Some of these

TABLE 3. *Measurements of Cerro Grande population*

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight	♂	27	45.39 ± 0.49	2.57	5.66
	♀	9	44.23 ± 0.87	2.62	5.92
Wing	♂	27	89.15 ± 0.36	1.89	2.12
	♀	9	83.36 ± 0.79	2.38	2.85
Tail	♂	26	101.39 ± 0.59	3.03	2.98
	♀	8	96.08 ± 1.16	3.27	3.40
Tarsus	♂	27	28.15 ± 0.16	.86	3.04
	♀	8	27.96 ± 0.44	1.24	4.43
Bill from nostril	♂	27	9.24 ± 0.09	.46	4.98
	♀	8	9.55 ± 0.19	.55	5.79

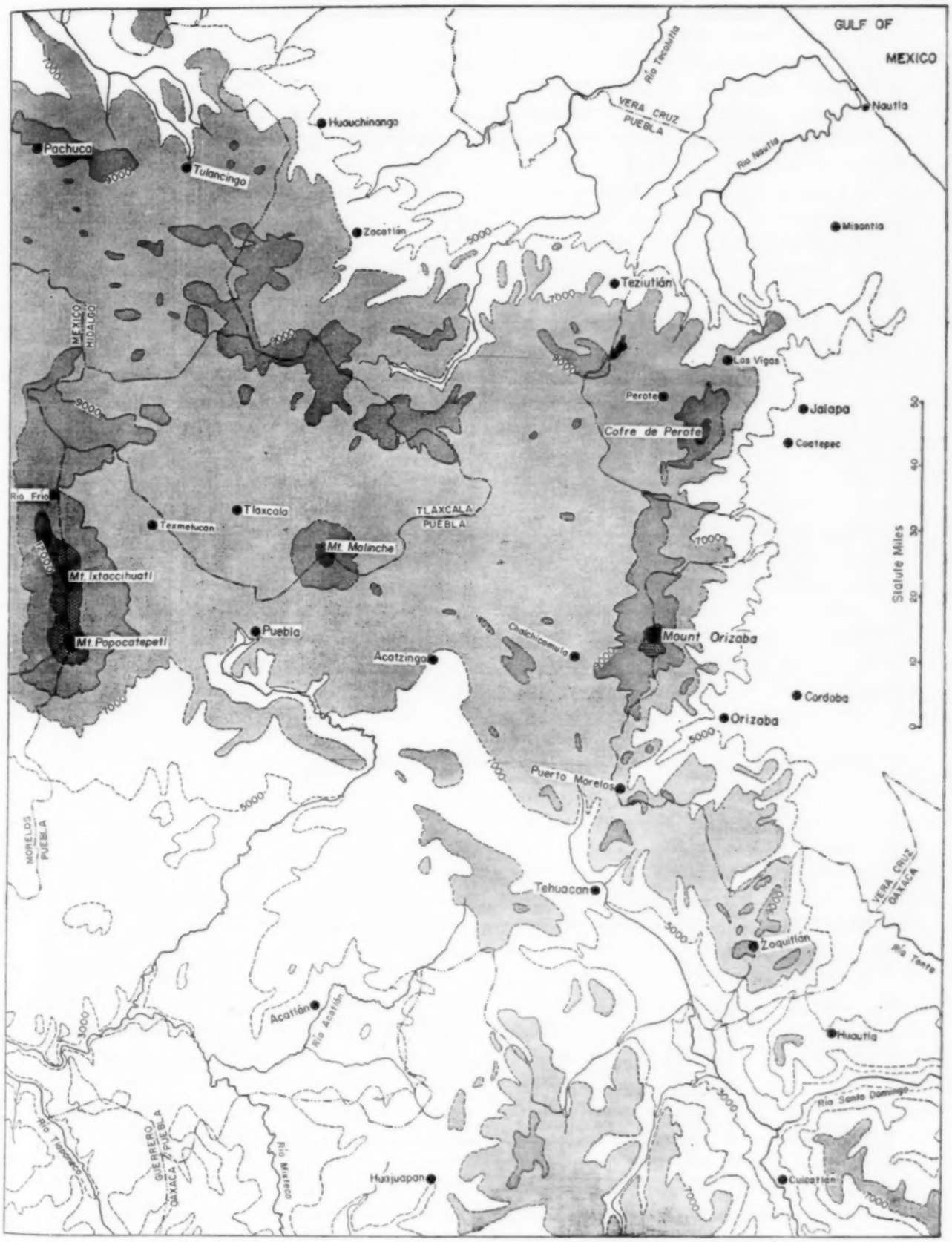

FIG. 5. The central eastern portion of the Mexican plateau indicating localities and their topographic relationships.

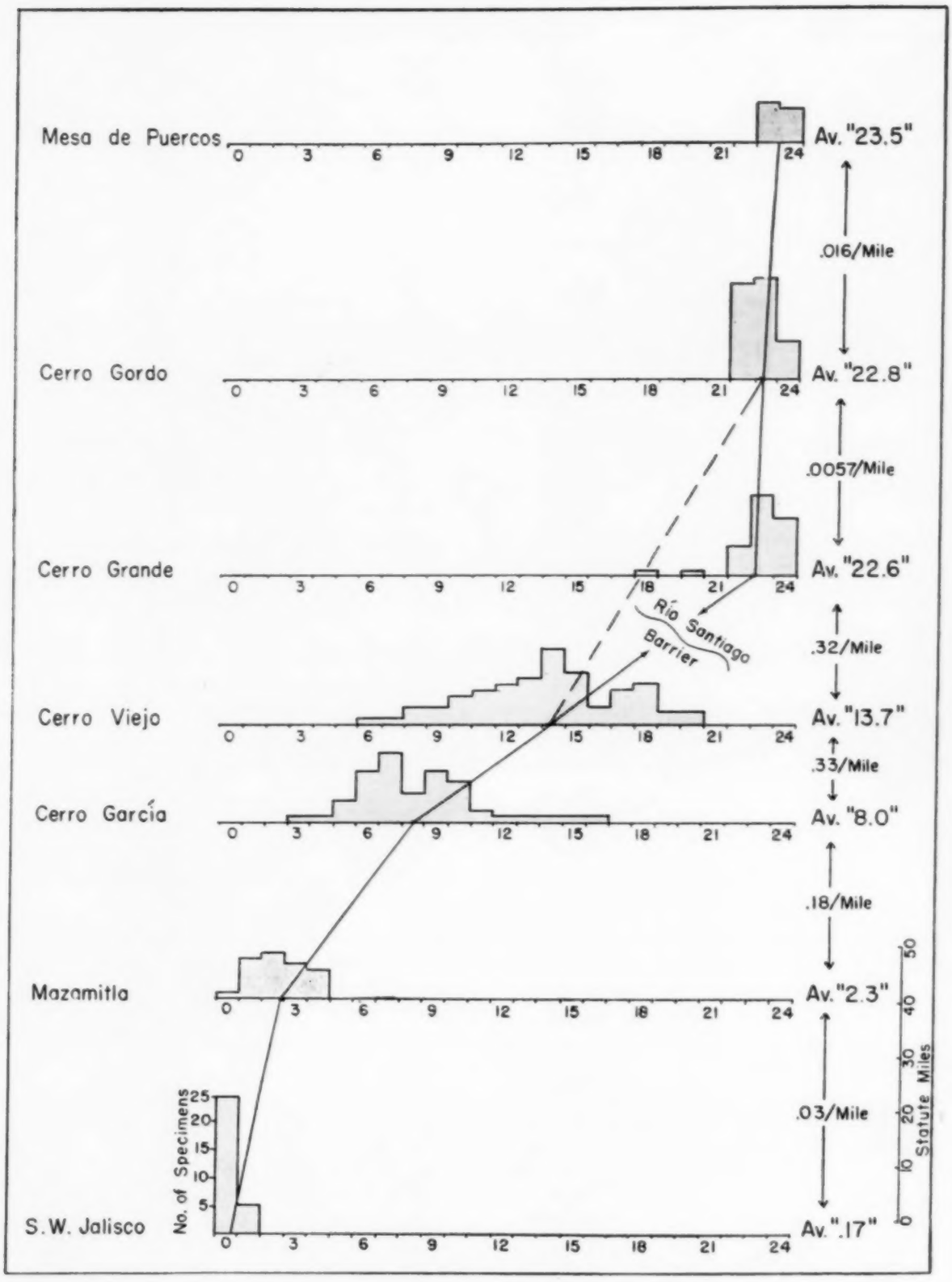

FIG. 6. Histograms of some hybrid populations in western Mexico. Average hybrid indices for population samples are enclosed in quotes. The change in hybrid index units on a per mile basis is indicated. Distance between base lines of the histograms is proportional to actual distance in air line miles between populations.

are over 6,000 feet in elevation and may support breeding populations of towhees. They certainly provide favorable avenues for the dispersal of juveniles and thus maintain a "filter bridge" between the Cerro Gordo and Cerro Grande populations. The existence of a favorable dispersal pathway between Cerro Gordo and Cerro Grande is further indicated by the extremely small shift in hybrid index which amounts to but .0057 hybrid index unit per mile.

Cerro Viejo

Located at the northwest end of Lake Chapala this mountain rises from an arid mesquite-grassland association at about 5,000 feet to 9,700 feet at the summit. Above 7,000 feet the dominant trees are oaks and madrones and near the summit dense thickets of a large woody *Salvia* provide excellent cover for towhees. A more extensive description and analysis of this locality was made in the previous paper (1950: 165–169).

The hybrid index, based on 76 specimens, ranges from "6" to "20" with the average at "13.7". This population is therefore 15 times more variable in color than a pure population of either of the parental species. No two of the 76 specimens are exactly alike (see 1950: figure 13 and plate 12).

The lowlands of the Río Santiago lie between Cerro Grande and Cerro Viejo (see figure 3). The river valley barrier is undoubtedly more effective than the minor discontinuities between Cerro Grande and Cerro Gordo. Proof of the relative effectiveness of the Río Santiago as a barrier is afforded by a comparison of the hybrid indices of the Cerro Viejo, Cerro Grande and Cerro Gordo populations. From "22.8" on Cerro Gordo the hybrid index changes only .2 of an index unit to "22.6" on Cerro Grande, over a distance of 35 miles. This is .0057 index unit per mile. From Cerro Grande ("22.6") to Cerro Viejo ("13.7") the change is 8.9 index units over an air line distance of 28 miles giving a change of .32 index unit per mile. If gene flow were equally free between these three populations we should expect the middle one, Cerro Grande, to have an index of approximately "17.7". For a graphic summary of these data see figure 6.

These relationships make it possible to assess the barrier value of the Río Santiago in terms of the shift it causes in the hybrid index of the Cerro Grande population. Assuming, that except for the barrier effect of the river, gene flow between Cerro Viejo and Cerro Grande would be equal to that between Cerro Grande and Cerro Gordo then the difference due to the river alone is 8.74 index units. Thus, the barrier of the Río Santiago and its lowlands is more than 50 times as effective as the 28 miles between Cerro Viejo and Cerro Grande would be if the river were not present and there was a moderately favorable habitat for dispersal between the two populations. This is an extremely crude estimate and it ignores other possible effects which may be present. It is nevertheless apparent that the Río Santiago and its associated lowlands are markedly more effective as a barrier than the ordinary plateau association.

In 1948, when the Cerro Viejo population was studied in the field, note was taken of possible routes of introgression from the populations of the parental species. The source of *ocai* genes seemed to be the Sierra de Tapalpa, 30 miles southwest of Cerro Viejo. It is now apparent that the closest source is from the birds on Cerro García, across the west end of Lake Chapala from Cerro Viejo. This situation will be treated in more detail in connection with the Cerro García hybrid population.

The source of the *P. erythrophthalmus* component of the Cerro Viejo hybrids was even less apparent in 1948. With the advantage afforded by hindsight it now seems that the guess which was made (1950: 169) in 1948 was correct, namely, that the *erythrophthalmus* influence on the Cerro Viejo population is via the series of mountain-top populations extending to

the northeast. The mountain noted as ". . . 13 miles east of Tepatitlán, Jalisco." (1950: 169) proved to harbor a towhee population (= Cerro Gordo) and is one of the "stepping-stones" operating to maintain the hybrid cline of which Cerro Viejo is a segment.

Cerro García

The village of San Luís Soyatlán located on the southwestern shore of Lake Chapala is at the north base of Cerro García. At the lake shore at 5,000 feet the vegetation is a semi-arid thorny scrub association which includes many cacti, mesquite and agave. The rise in elevation is rapid and the first good towhee cover is reached at approximately 7,000 feet. Above 8,000 feet the dominant trees are oaks and madrones with abundant brushy understory. Near the summit, which is 9,044 feet in elevation, there are thickets of the same *Salvia* which furnishes such excellent cover for towhees on Cerro Viejo and the birds were equally abundant. Figure 14 is a photograph taken from approximately 8,500 feet altitude looking northeast across Lake Chapala.

The hybrid index, based on 58 specimens, ranges from "3" to "16" with the average at "8.0". The Cerro García population therefore possesses nearly the same magnitude of variability as the Cerro Viejo population although, as would be expected, it is closer to *ocai* in complexion. It is significant that these two populations which are closest to the midpoint of the hybrid index scale also show the greatest number of recombination types. This is what would be expected if the variability is due to introgressive hybridization. (Table 4 contains data on mensural characters.)

The west end of Lake Chapala lies between Cerro Viejo and Cerro García. Extending northwest from Cerro García is a ridge which rises slightly above 7,000 feet. It is covered with an arid thorn scrub and does not appear favorable as breeding habitat for towhees but probably acts as a dispersal pathway for juveniles. There is a low gap which drops to 5,000 feet in altitude between the north end of this ridge and Cerro Viejo. This gap would increase the barrier effect. The map (figure 3) indicates the relationships of Cerro García and Cerro Viejo.

South of Cerro García is a valley 3–8 miles wide in which the town of Teocuitatlán is situated. This valley is well below 5,000 feet in elevation and connects to the west with the lowlands at the headwaters of the Río Ameca. East of Cerro García the barrier toward the Mazamitla region is less complete but it is partial. There is a considerable gap in favorable towhee habitat which connects from the Teocuitatlán area to Lake Chapala and serves to isolate Cerro García from direct contact with the Mazamitla population.

TABLE 4. *Measurements of Cerro García population*

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight	♂	27	51.94 ± 0.56	2.92	5.62
	♀	26	50.10 ± 0.59	2.99	5.96
Wing	♂	27	87.73 ± 0.47	2.45	2.79
	♀	26	83.91 ± 0.52	2.64	3.15
Tail	♂	26	100.96 ± 0.77	3.93	3.89
	♀	20	96.80 ± 0.69	3.09	3.19
Tarsus	♂	27	29.73 ± 0.24	1.26	4.23
	♀	25	29.26 ± 0.21	1.06	3.62
Bill from nostril	♂	26	9.40 ± 0.08	.44	4.65
	♀	26	9.45 ± 0.09	.49	5.17

TABLE 5. *Measurements of Mazamitla population*

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight	♂	13	56.92 ± 0.80	2.90	5.09
	♀	14	52.23 ± 0.87	3.25	6.22
Wing	♂	13	88.65 ± 0.72	2.61	2.94
	♀	14	84.44 ± 0.66	2.47	2.92
Tail	♂	12	100.62 ± 1.71	5.91	5.87
	♀	14	96.95 ± 1.73	6.47	6.67
Tarsus	♂	13	30.53 ± 0.27	.99	3.26
	♀	14	30.14 ± 0.26	.99	3.30
Bill from nostril	♂	13	10.12 ± 0.21	.75	7.46
	♀	13	9.80 ± 0.12	.43	4.41

Mazamitla

Looking south from the summit of Cerro García, beyond the valley of Teocuitatlán, one sees a series of east-west ridges which rise above 7,000 feet and show the dark green of coniferous forest on their summits. To the east in this same series of ridges, is the town of Mazamitla. At 4 miles west of Mazamitla, 6,800 feet elevation, the vegetation is pine-oak with hawthorne thickets bordering the roads and fields. The general appearance of the vegetation is similar to that in the Sierra de Tapalpa. Figure 15 illustrates the general aspect of the vegetation and topography.

Towhees were not abundant at this locality. They occurred in the hawthorne thickets and in hedgerows bordering cornfields. The 29 specimens ranged from "0" to "4" with an average hybrid index of "2.3". See table 5 for measurements.

Because no specimens were then available this area was indicated by a large "question-mark" on the map (figure 16, p. 170) in the 1950 paper. It is interesting to find that its average ("2.3") falls at exactly the proper place on the line connecting the averages of the two adjacent populations on the graph showing the trend of color in the transplateau gradient (1950: figure 18).

To the west of Mazamitla the trend toward *P. ocai alticola* continues to the Sierra de Tapalpa population with an average hybrid index of ".17". The barrier between is the broad, arid valley of the Sayula-Ciudad Guzmán lowlands. In most parts this is below 4,000 feet in elevation and is undoubtedly highly effective as a barrier to the dispersal of towhees.

To the east the barrier between the Mazamitla birds and those of the Cerro Tancítaro highlands (average index "4.0") is probably less effective. There are no areas below 5,000 feet and there are many small mountains which rise above 7,000 feet which probably support breeding populations of towhees.

Figure 11 is a pictorialized scatter diagram of some of the populations in the introgressing series from La Mesa de los Puercos to Mazamitla. Cerro Gordo was not included because of the similarity of its population to that of Cerro Grande. The weights of Cerro Viejo specimens were not taken so they cannot be plotted but it is apparent that the Cerro Viejo birds would fall in the area indicated. The correlation between weight and color pattern is well demonstrated by this diagram. The effects of introgression on color and size are equally apparent.

The second introgressive cline to be considered is that which includes the population of La Mesa de los Puercos, Cerro

Gordo, Cerro El Fraile, Las Joyas, and the Tancítaro-Pátzcuaro region.

The characteristics of the La Mesa de los Puercos and Cerro Gordo populations have been discussed on p. 00.

Cerro El Fraile

This mountain rises from an altitude of 6,400 feet at the base to nearly 8,800 feet at the summit. The vegetation below 7,000 feet is mainly a semi-arid thorny scrub although scattered oaks come down to the base of the mountain. Optimum towhee habitat is found from 7,500 feet to the summit and consists of an oak-madrone woodland with a dense understory of *Baccharis, Ceanothus,* and other shrubs. Tuna cactus occurs to the summit. We found no pines on the mountain and the local inhabitants told me that pines were not known on El Fraile although a few were said to occur on the Cerro de Zináparo, six miles to the northeast. The nearest large stands of pines are on the mountains east and west of the town of Purepero, about 12 miles south of Cerro El Fraile. These pines are the northernmost large stands in this area and apparently there are no large stands of pines between Purépero and the area near La Mesa de los Puercos, over 100 miles to the northwest in an air line. The map (figure 4) shows the relationships of the topography in the vicinity of Cerro El Fraile. Figure 16 is a photograph taken from near the summit of Cerro El Fraile looking toward the southeast in the direction of Las Joyas.

The hybrid population inhabiting this mountain proved the most unpredictable of those investigated. Judging from its proximity to the Tancítaro-Pátzcuaro region it would be expected to show strong affinities to *ocai*. However, its average hybrid index, based on 13 adult specimens, proved to be "21.7". One specimen each was scored for "11", "21", and "24". Four were scored "22" and six were scored "23". The single "11" individual possibly indicates that this sample is too small to show the true shape of the curve of color variation. It is probable that the average would shift slightly downward if a larger sample was available. (See table 6 for measurements of this population.)

From Cerro Gordo to Cerro El Fraile is a distance of 55 miles in an air line. The lowlands of the Río Lerma lie between but do not fall below 5,000 feet in elevation. There are several oak-covered hills between Cerro Gordo and Cerro El Fraile which almost certainly harbor breeding populations of towhees. The shift in hybrid index between these two localities is relatively small, being but "1.1" index units in the 55 miles or .02 hybrid index unit per mile. The change is thus of the same order of magnitude as that beween La Mesa de los Puercos and

TABLE 6. *Measurements of Cerro El Fraile population*

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight	♂	9	42.98 ± 0.98	2.93	6.81
	♀	4	40.22 ± 1.55	3.11	7.73
Wing	♂	9	88.37 ± 0.57	1.73	1.95
	♀	4	82.05 ± 1.11	2.22	2.70
Tail	♂	9	101.80 ± 1.26	3.78	3.71
	♀	4	93.47 ± 1.09	2.19	2.34
Tarsus	♂	8	28.17 ± 0.29	.83	2.94
	♀	4	27.70 ± 0.32	.63	2.29
Bill from nostril	♂	9	9.55 ± 0.17	.50	5.28
	♀	4	9.55 ± 0.33	.45	4.71

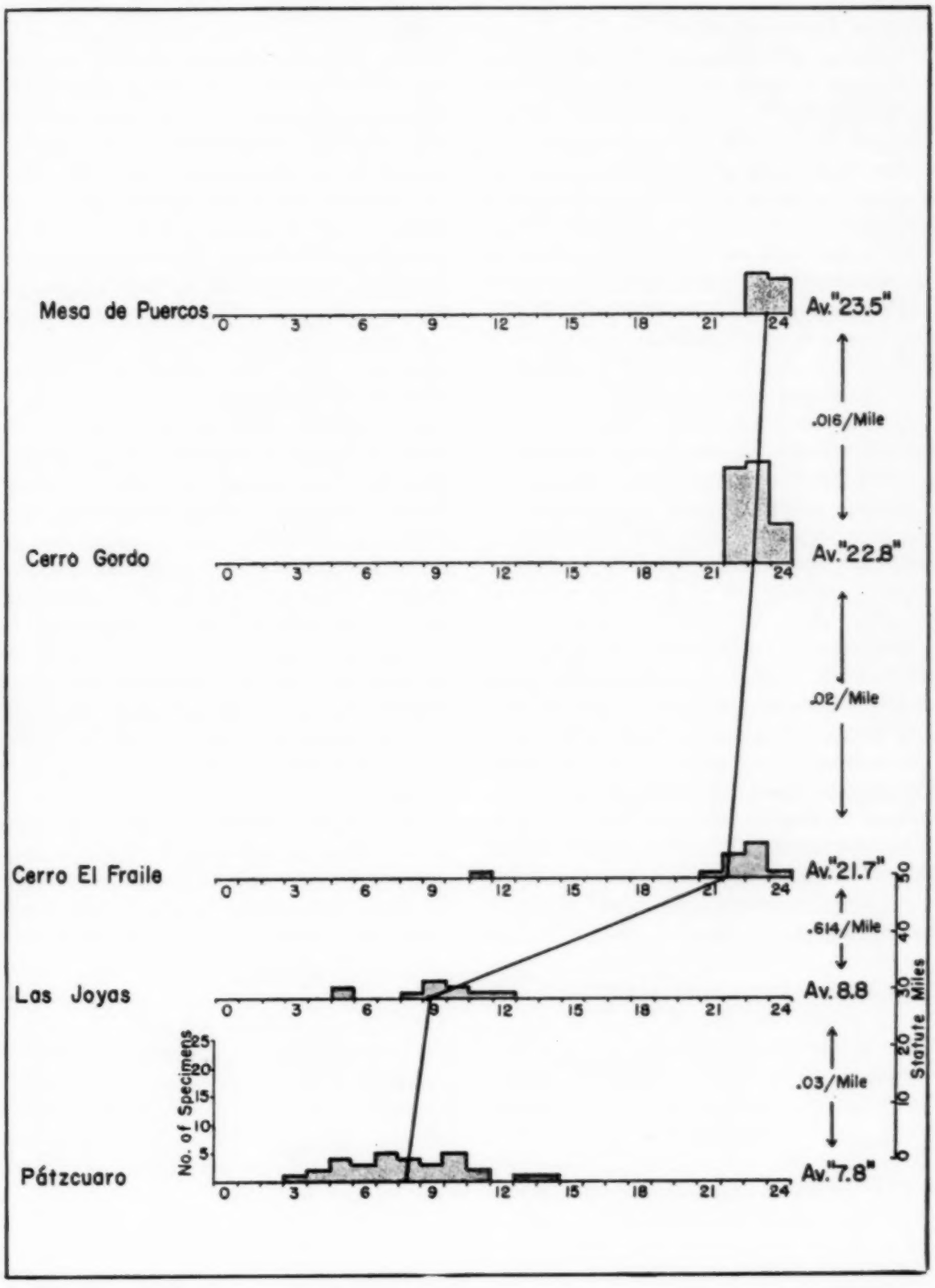

FIG. 7. Histograms of some hybrid populations in western Mexico. See figure 6 for explanation of this graph.

Cerro Gordo where it is .016 unit per mile.

From Cerro El Fraile to Las Joyas, 21 miles to the southeast, the shift in hybrid index is large and abrupt. The Las Joyas population has an average hybrid index of "8.8". This is a change of .614 hybrid index unit per mile, or more than 30 times as great as the per mile change from Cerro Gordo to Cerro El Fraile. This is the most rapid hybrid gradient so far discovered in these birds. Between Las Joyas and the Lake Pátzcuaro region the change is again gradual being but .03 hybrid index unit per mile. Figure 7 portrays these relationships.

Before developing an explanation for this situation it is necessary to describe the ecological situation at Las Joyas.

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Las Joyas

This locality is 12 miles west of Zacapu, Michoacan, and a few hundred yards south of the main highway from Mexico City to Guadalajara. The elevation in the vicinity varies from 7,500 to 8,000 feet and the topography is broken, rugged, and of volcanic origin. The principal trees are pines with oaks, madrones and alders of secondary importance. There is no tuna cactus at Las Joyas although it occurs from the base to the summit on El Fraile. All aspects of the vegetation indicate a higher rainfall and lower temperature at Las Joyas than on Cerro El Fraile. This is substantiated by the official rainfall and temperature figures for the past five years. At Pátzcuaro the five-year average temperature is 61 degrees F. and the rainfall has averaged 44 inches. At Guadalajara these figures are 66 degrees F. and 37 inches and at León, 65 degrees F and 26 inches. The vegetation from Cerro El Fraile north to Cerro Gordo is intermediate in general aspect between the vegetative environments at Guadalajara and León. The rainfall and temperature figures may also be assumed to be approximately intermediate, namely, 65 degrees F. and 32 inches. It is apparent that the environment at Cerro El Fraile is more like that on Cerro Gordo than that at Las Joyas. That this environment apparently favors *erythrophthalmus* more than *ocai* is indicated by the widespread habitat preference of *erythrophthalmus* for an oak association as compared with the conifer association preference of *ocai*. Figure 17 illustrates the aspect of the vegetation at Las Joyas. Table 7 contains the measurements of the Las Joyas birds.

In view of the above evidence it seems most logical to conclude that the strongly *erythrophthalmus*-like complexion of the El Fraile population is due to the favorable environment for *erythrophthalmus*

TABLE 7. *Measurements of Las Joyas population*

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight	♂	6	53.60 ± 1.72	4.22	7.87
	♀	4	46.6 ± 1.13	2.27	4.87
Wing	♂	6	90.28 ± 0.84	2.07	2.29
	♀	4	82.07 ± 1.26	2.53	3.08
Tail	♂	3	106.80 ± 2.09	3.63	3.39
	♀	1	(90.8)		
Tarsus	♂	6	30.70 ± 0.21	.51	1.64
	♀	4	29.67 ± 0.32	.64	2.15
Bill from nostril	♂	6	9.83 ± 0.24	.58	5.87
	♀	4	9.60 ± 0.21	.42	4.41

TABLE 8. *Measurements of Cerro Tequila population*

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight	♂	35	46.95 ± 0.51	2.99	6.37
	♀	25	45.86 ± 0.83	4.16	9.07
Wing	♂	35	87.96 ± 0.42	2.48	2.82
	♀	25	83.32 ± 0.53	2.65	3.18
Tail	♂	35	100.47 ± 0.67	3.96	3.94
	♀	25	96.21 ± 0.72	3.60	3.74
Tarsus	♂	35	28.72 ± 0.13	.75	2.61
	♀	25	28.02 ± 0.19	.94	3.34
Bill from nostril	♂	35	9.6 ± 0.08	.46	4.78
	♀	25	9.6 ± 0.08	.38	3.97

which exists on Cerro El Fraile. It is probable that El Fraile was originally colonized by towhees from the north. These pioneers were probably no more tainted by introgression than the Cerro Gordo birds. The more *ocai*-like birds of the Pátzcuaro-Tancítaro population had not become established on El Fraile because, for them, the habitat was unfavorable. Since the establishment of the population of almost pure *erythrophthalmus* on El Fraile, there has been enough introgression from the Las Joyas region to shift the average hybrid index slightly toward *ocai*. Similarly, the effect of introgression from El Fraile to Las Joyas is indicated by the Las Joyas average index of "8.8" as compared with the "7.8" average at Pátzcuaro and the "4.0" average at Mount Tancítaro.

The third introgressive gradient is that which includes the populations of *erythrophthalmus* north of the Río Santiago, Cerro Tequila, Sierra de Ameca, and the populations of *ocai* south of the Río Ameca. The two terminal populations have already been described.

Cerro Tequila

Rising from a base level of 4,000 feet the volcanic peak of Cerro Tequila reaches an altitude of nearly 10,000 feet at the summit. At the village of Sandovale at 4,600 feet on the north slope, the vegetation is a thorny scrub including cactus, agave and mesquite. At 5,400 feet the first pines and oaks are encountered and at 6,000 feet there is a pine, oak and madrone woodland with a brushy understory. From 6,500 feet to the summit towhees are abundant in suitable habitat which is optimum in the dense undergrowth of steep-sided arroyos. Figure 18 illustrates the topographic relationship between Cerro Tequila and the surrounding lowlands.

The 69 specimens from Cerro Tequila range from "10" to "23" with an average hybrid index of "18.0". (See table 8 for mensural data.)

From Cerro Tequila to the nearest possible source of "pure" *erythrophthalmus* is approximately 24 miles in an air line. The deep gorge of the Río Santiago, which drops to 2,000 feet at this point, greatly increases the barrier effect of the distance. It may be assumed that a bird as highly adapted to living in dense thickets as is a towhee, would seldom, if ever, fly directly across such a barrier. Far more likely, individuals which cross it do so by working gradually down one side of the canyon, fly the irreducible minimum of the river's width and work up the opposite side keeping to the thickets.

Extending northwest from Cerro Tequila is a tongue of oak woodland which connects with a high mesa approximately

10 miles away on the edge of the Río Santiago gorge. This mesa, known as Cerro Viejo de Magdalena, was visted on September 12, 1950, by J. R. Alcorn, A. C. Browne and E. A. Olson. The oaks were sparse and ground cover was poor. No towhees were found. Another such mountain located 14 miles WNW of Guadalajara was visited by the same persons on September 9, 1950. This mountain reached 6,625 feet and supported a sparse cover of oaks and a few pines. As on Cerro Viejo de Magdalena, no towhees were found.

On August 19, Browne, Olson and Sibley explored a mountain called Cerro San Miguel located approximately 11 miles southwest of Guadalajara. At 5,900 feet the vegetation was sparse pine and oak woodland with very little ground cover. No towhees were found here either.

It is apparent from these three situations that the presence of towhees in southwestern Mexico depends upon abundant, dense, low vegetation, regardless of the presence of pine-oak woodland and that areas of suitable habitat are not likely to be found on relatively small mountains which do not reach well above 7,000 feet in elevation.

Sierra de Ameca

Rising sharply north of the town of Ameca at 4,000 feet is this small range which reaches 8,800 feet at the highest point. The vegetation around the base is arid sub-tropical in general character with mesquite and cactus on the lower slopes. Sugar cane is grown in the valley of the Río Ameca.

Our ascent of the Sierra de Ameca was made from Ahualulco de Mercado on the north side. At 5,600 feet the vegetation is subtropical deciduous woodland and dense thorny scrub. Above 6,000 feet the principal cover is a pine, oak and madrone woodland with brushy understory vegetation in the ravines. Figure 19 depicts the north side of the Sierra de Ameca. Towhees were decidedly uncommon and the exceptionally steep topography made hunting so difficult that only 18 specimens were obtained by four collectors in four days. These ranged from "7" to "19" with an average hybrid index of "12.0". Table 9 contains data on measurements.

From Cerro Tequila to the Sierra de Ameca is a distance of 16 miles. The intervening country is approximately 4,000 feet in elevation and is entirely unfavorable for towhees. To the southwest the nearest known population of *P. ocai alticola* is approximately 35 miles away on the other side of the Río Ameca. There may be small populations of *P. o. alticola* as near as 20 miles but specimens are not known.

The hybrid cline which includes Cerro

TABLE 9. *Measurements of Sierra de Ameca population*

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight	♂	14	48.10 ± 0.66	2.45	5.09
	♀	4	48.45 ± 0.46	.93	1.91
Wing	♂	14	88.61 ± 0.79	2.97	3.35
	♀	4	83.0 ± 1.61	3.22	3.87
Tail	♂	14	102.27 ± 1.37	5.12	5.00
	♀	4	94.67 ± 1.68	3.38	3.57
Tarsus	♂	14	29.21 ± 0.25	.93	3.17
	♀	4	29.25 ± 0.56	1.11	3.79
Bill from nostril	♂	14	9.31 ± 0.14	.53	5.74
	♀	4	9.85 ± 0.17	.34	3.47

Tequila and the Sierra de Ameca is remarkably smooth in its trend from one parental species, through the two hybrid populations, to the other parental species. Figure 8 illustrates the situation graphically. The combination of isolated "islands" of suitable habitat on the mountain tops coupled with the Río Ameca and Río Santiago barriers have acted to establish and maintain this hybrid gradient.

Cerro El Faro (Llorón de Ixtlán)

Another mountain located between the Río Ameca and the Río Santiago is Cerro El Faro, six miles south of the town of Ixtlán del Río, Nayarit (see figure 2). At the town the elevation is 3,350 feet. Suitable towhee habitat occurs above 6,500 feet with the optimum habitat between 7,000 feet and the summit at 7,635 feet. Above 6,500 feet the vegetation is dominated by pines, oaks and madrones with a few thorny shrubs on flat areas and dense thickets of shrubs and herbaceous plants in the ravines.

The 26 adult specimens ranged from "8" to "22" with an average hybrid index of "15.8". Figure 8 includes the graphic analysis of this population and table 10 presents the measurements.

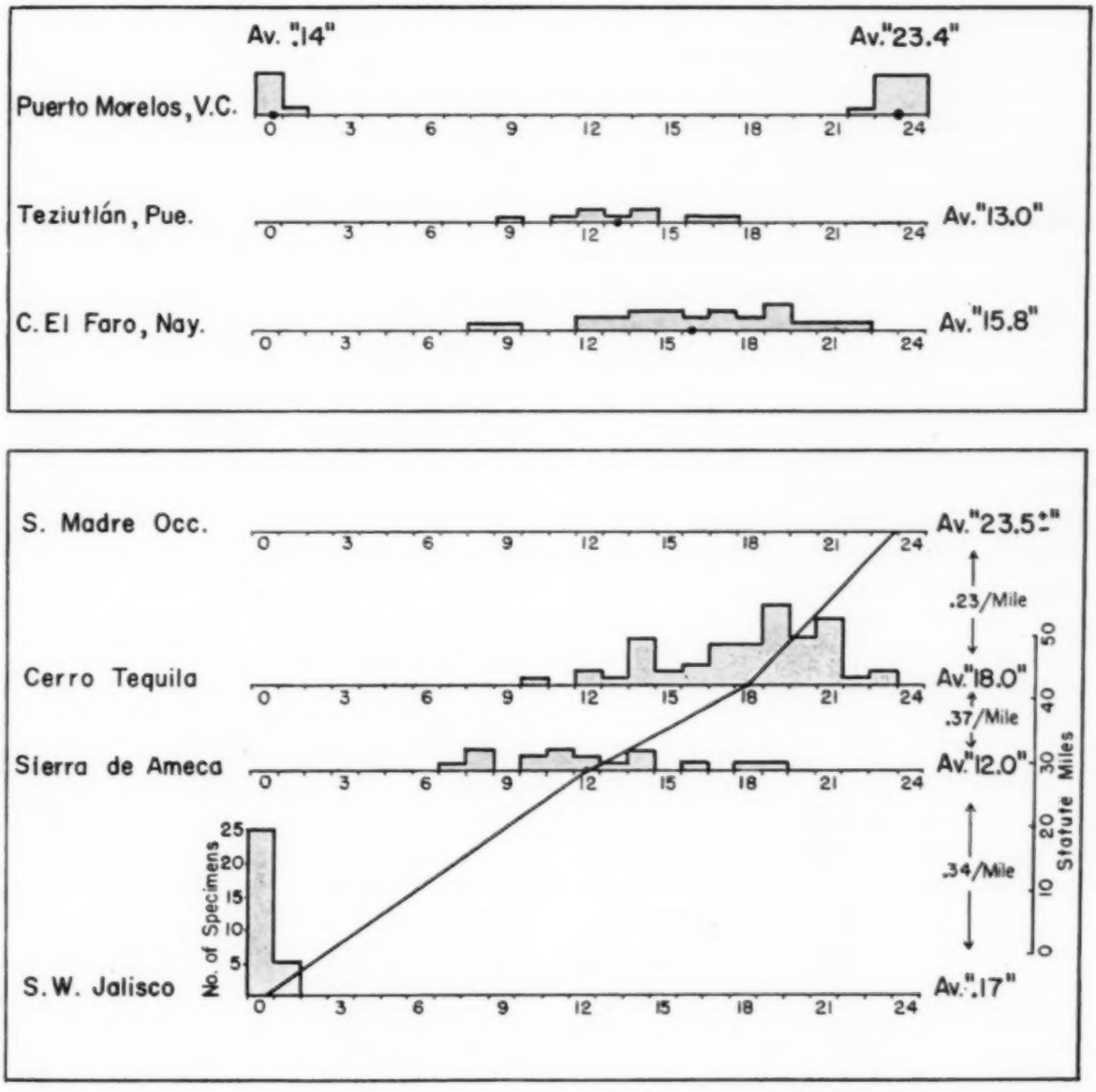

FIG. 8. Histograms of some hybrid populations of red-eyed towhees in Mexico. See figure 6 for explanation of this graph.

TABLE 10. *Measurements of Cerro El Faro population*

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight	♂	10	50.90 ± 1.21	3.83	7.52
	♀	14	48.58 ± 0.59	2.20	4.52
Wing	♂	11	89.34 ± 0.79	2.62	2.93
	♀	15	83.99 ± 0.35	1.34	1.59
Tail	♂	8	103.53 ± 1.48	4.21	4.06
	♀	5	97.72 ± 1.00	2.23	2.28
Tarsus	♂	11	29.14 ± 0.30	1.00	3.43
	♀	13	28.67 ± 0.24	.87	3.03
Bill from nostril	♂	11	9.80 ± 0.16	.54	5.48
	♀	15	9.57 ± 0.12	.46	4.79

In general ecological aspect this locality appears to be more favorable for *P. erythrophthalmus* than for *P. ocai*. Also, as with some of the other hybrid localities (e.g., Cerro El Fraile) it is likely that the first pioneers to colonize El Faro were from the north. This seems likely because *P. erythrophthalmus* should be better able to cross lowland areas since it is adapted to lower elevations than is *P. ocai*.

THE EASTERN PLATEAU POPULATIONS

Specimens of interspecific hybrids between *Pipilo erythrophthalmus* and *P. ocai* have been taken at several localities along the eastern edge of the Mexican plateau. The earliest of these was the type of *Pipilo torquata* Du Bus (1847: 105) which probably came from the north slope of the Cofre de Perote near Las Vigas, Vera Cruz (Sibley, 1950: 144). This specimen was collected at least 109 years ago. In addition to this specimen there have been 15 others out of a total of 115 from the Mount Orizaba-Cofre de Perote region which have shown evidence of hybridization. Two hybrid specimens taken near Teziutlán, Puebla in 1884 were both described as "new species" by Ridgway (1886: 332). These specimens and the ecological situation on Mount Orizaba were described in the 1950 paper, pp. 155–165.

TABLE 11. *Measurements of Teziutlán population*

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight	♂				
	♀				
Wing	♂	3	82.96 ± 0.69	1.20	1.44
	♀	6	79.35 ± 0.55	1.36	1.71
Tail	♂	2	93.60 ± 0.70	.99	1.05
	♀	5	93.14 ± 2.13	4.77	5.12
Tarsus	♂	3	29.93 ± 0.56	.97	3.24
	♀	6	28.66 ± 0.27	.66	2.32
Bill from nostril	♂	3	10.30 ± 0.11	.20	1.94
	♀	6	9.80 ± 0.18	.43	4.41

Teziutlán

Teziutlán, Puebla was visited September 7–9, 1950. In an area known locally as "Zaquiolca," located three miles air line west of Teziutlán at 7,300 feet elevation, 11 specimens were collected. All of these are hybrids, ranging from "9" to "17" with an average hybrid index of "13.0". The dominant trees are pines and oaks and the hybrids were found in thickets of low, dense *Alnus* and *Baccharis*. See figure 8 for the graphic analysis of these hybrids and table 11 for their measurements.

Two additional specimens representing this hybrid population were collected at "Rancho San Miguel, Kilometer 347, Vera Cruz" by E. and M. Traylor. This locality is approximately eight miles (air line) northeast of Teziutlán on the road to Nautla. It is less than two miles from the border between Puebla and Vera Cruz and at an altitude of approximately 5,000 feet. Beyond this point the edge of the plateau drops sharply away and it is unlikely that suitable towhee habitat continues much below this point.

The two specimens are deposited in the Chicago Natural History Museum. No. 187573 (♂ immature, collected August 30, 1948) is in the post-juvenal molt but the color areas can be determined. It has a hybrid index of "15". No. 187574 (♀, collected August 31, 1948) has a hybrid index of "16". Thus these specimens fit into the pattern of this population and indicate that the birds occur at least as low as 5,000 feet in the Teziutlán area.

From the meagre evidence available previous to my visit it had been assumed that the situation at Teziutlán was similar to that on the west slope of Mount Orizaba, namely, sympatric hybridization with both parental species present. It is now apparent that the situation at Teziutlán is actually that of a partially isolated hybrid population, similar to those which have been found at such localities as Cerro Viejo, Cerro García and Cerro Tequila in southwestern Mexico.

The extent of the area occupied by the Teziutlán population and the barriers which have permitted its establishment are incompletely known. Judging from what is known of the distribution of favorable habitat it seems likely that the area occupied is approximately as indicated on the map, figure 1. The rapid drop in elevation at the edge of the plateau is the barrier to the north and northwest. To the east and southeast, between Teziutlán and Perote, the country is quite open and favorable cover is uncommon. The rain shadow of the Cofre de Perote has permitted the development of an extensive, open grassland which partially isolates the Teziutlán area to the south and there is a deep barranca on the west which is the probable barrier between the Teziutlán birds and the populations of *P. erythrophthalmus maculatus* which occur in Hidalgo and northwestern Puebla.

The existence of the Teziutlán hybrid population depends upon the presence of this "island" of favorable habitat, partially isolated from other towhee populations and situated between populations of the two parental species, *erythrophthalmus* to the northwest and *ocai* to the southeast.

Puerto Morelos

A second locality on the east edge of the plateau was visited on September 10 and 11, 1950. At Puerto Morelos, 19 miles westsouthwest of Orizaba, Vera Cruz, the highway from Tehuacán, Puebla to Orizaba, Vera Cruz reaches its highest elevation at 8,000 feet. Puerto Morelos is 20 miles south of the hybrid zone above Chalchicomula on the west slope of Mount Orizaba (1950: 155–165).

At Puerto Morelos the vegetation is oak woodland with *Baccharis* thickets on the open hillsides. Both species of towhees were found at Puerto Morelos. Although *erythrophthalmus* seemed the more abundant, *ocai* was present in good numbers. The two species were observed in the same thickets and were as completely sympatric as they were on Cerro San Felipe, Oaxaca (1950: 152–155) and on the west slope of Mount Orizaba (1950: 155–165). Fifteen specimens were col-

TABLE 12. *Measurements of Puerto Morelos population*, Pipilo erythrophthalmus

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight					
Wing	♂	6	84.23 ± 0.64	1.56	1.85
	♀	5	79.60 ± 0.70	1.55	1.94
Tail	♂	4	96.47 ± 1.24	2.47	2.56
	♀	5	91.40 ± 1.08	2.43	2.65
Tarsus	♂	5	29.24 ± 0.52	1.16	3.96
	♀	5	27.84 ± 0.19	.42	1.49
Bill from nostril	♂	6	10.42 ± 0.19	.47	4.52
	♀	5	10.12 ± 0.07	.17	1.65

lected, 11 *P. erythrophthalmus* and 4 *P. ocai*. In addition 3 *ocai* and 2 *erythrophthalmus* in adult plumage were shot for me by Panuncio Rosas, a resident of Puerto Morelos. These latter birds were too badly damaged to save as study skins but they were carefully examined and their plumage characters were recorded.

In the 13 specimens of *P. erythrophthalmus* there are two which show chestnut shaft streaks on the crown ("3") and 7 which show some white ("3") in the throat. The average hybrid index for these 13 birds is "23.4". (See table 12 for the measurements of the 11 individuals saved as study skins.)

The seven specimens of *P. ocai* show even less evidence of introgression. Only one bird has a few black feathers in the throat. Otherwise they appear to be "pure" *P. o. ocai*. The average hybrid index for this small sample is ".14". Figure 8 includes the graphic analysis of these specimens and table 13 contains their measurements. It is apparent that these few specimens do not adequately portray the entire picture at this locality but they indicate that a hybrid population is not present and that introgression is relatively small in amount.

Zoquitlán

A further clue to the complexity of the problem in this region was discovered in the Museo Nacional de la Historia Natural in Mexico City. Through the courtesy

TABLE 13. *Measurements of Puerto Morelos population*, Pipilo ocai

Item	Sex	Number of specimens	Mean with standard error	Standard deviation	Coefficient of variation
Weight					
Wing	♂	1	(86.50)		
	♀	3	82.43 ± 2.19	(not reliable) .38	4.62
Tail	♂				
	♀	1	(96.7)		
Tarsus	♂	1	(32.0)		
	♀	3	31.57 ± 2.19	(not reliable) .38	1.20
Bill from nostril	♂	1	(11.1)		
	♀	3	11.13 ± 0.07	.12	1.09

of Prof. Liborio Martinez I was permitted to examine the towhees in the collection under his care. Three specimens, labelled "Zoquitlán, Puebla," were studied. One of these, which seemed to be a pure *P. ocai,* was mounted alone on a pedestal display perch. The other two were mounted side by side on another pedestal with the number "2465" indicated as a catalog number. One of these was seemingly a pure *P. erythrophthalmus* while the other was an obvious hybrid with the following characters: Pileum black ("4"); dorsal spotting absent ("0"); back color green with black streaks ("1"); throat black ("4"); flanks rufous ("4"); tail spots reduced and clouded with greenish ("2"). These give a summated hybrid index of "15" for this bird. Further evidence of hybridization is indicated by yellow at the bend of the wing and a short white loreal line both of which are characteristic of *P. ocai* but not of *P. erythrophthalmus*.

If the locality data for these three specimens are correct they indicate that at Zoquitlán there is an area of sympatric hybridization which may be similar to the situation on the west slope of Mount Orizaba. Further study of this population is needed.

The Río Santo Domingo Barrier

Twenty-five miles south of Zoquitlán and 4,000 feet lower in elevation is the Río Santo Domingo. The lowlands associated with this large river cut a wide transverse swath through the mountains and act to isolate the towhee populations of Zoquitlán and the Mount Orizaba region from those in the Cerro San Felipe —Cerro Zempoaltepec uplands to the south in Oaxaca. The maps, figures 1 and 5, indicate these topographic relationships. The barrier effect to towhees is increased by the highly unfavorable environment of the valley of the Río Santo Domingo and its tributaries. Goldman (1951: 220) describes the valley of the Río Santo Domingo as a "great chasm" which "bisects the mountain range and carries the united streams flowing from the valleys at Tehuacán, Puebla, at the north, Cuicatlán on the south, and other interior drainage east to the Gulf. Beyond this chasm a high, pine-covered ridge extends north along the eastern border of the table land of Puebla to near the gap just south of Mount Orizaba." This "pine-covered ridge" is that on which Zoquitlán is located.

The valley in which Tehuacán, Puebla is situated is described by Goldman (1951: 237) as "nearly level in cross section and has a solid limestone floor, . . . From the lower end of the valley the drainage is to the Río San Domingo, a branch of the Río Papaloapán. The valley is bordered on both sides by limestone hills which rise abruptly to low mountains a few miles away. The valley near Tehuacán is very arid and barren, but all the surrounding hills are covered with a remarkably luxuriant growth of desert plants." North of Tehuacán the desert, created by the rain shadow of the Mount Orizaba massif, continues and effectively isolates the highlands of the Mount Orizaba region from those to the west (Mount Malinche, etc.). It is apparent that the Río Santo Domingo, its associated lowlands, and the rain-shadow desert west of Mount Orizaba provide an effective barrier to the dispersal of towhees between the Oaxaca highlands and the Mount Orizaba-Zoquitlán populations. It is undoubtedly this barrier which has prevented gene exchange between these populations and thus introgression has not been able to lower the physiological or ethological threshold in either species on Cerro San Felipe and thereby promote hybridization between them. This seems to be a logical explanation for the existence of the completely sympatric populations which occur south of the Río Santo Domingo in Oaxaca.

As now known, the relationships between the two parental species in the mountain chain which runs along the eastern edge of the Mexican plateau from Oaxaca to Hidalgo may be summarized as follows:

1. Sympatric in Oaxaca with no evidence of introgression indicated in 21 specimens of *P. e. oaxacae* and 43 of *P. o. brunnescens* (Sibley, 1950: 152–155).
2. An occasional hybrid formed between the sympatric parental species at Zoquitlán, Puebla. (Evidence incomplete.)

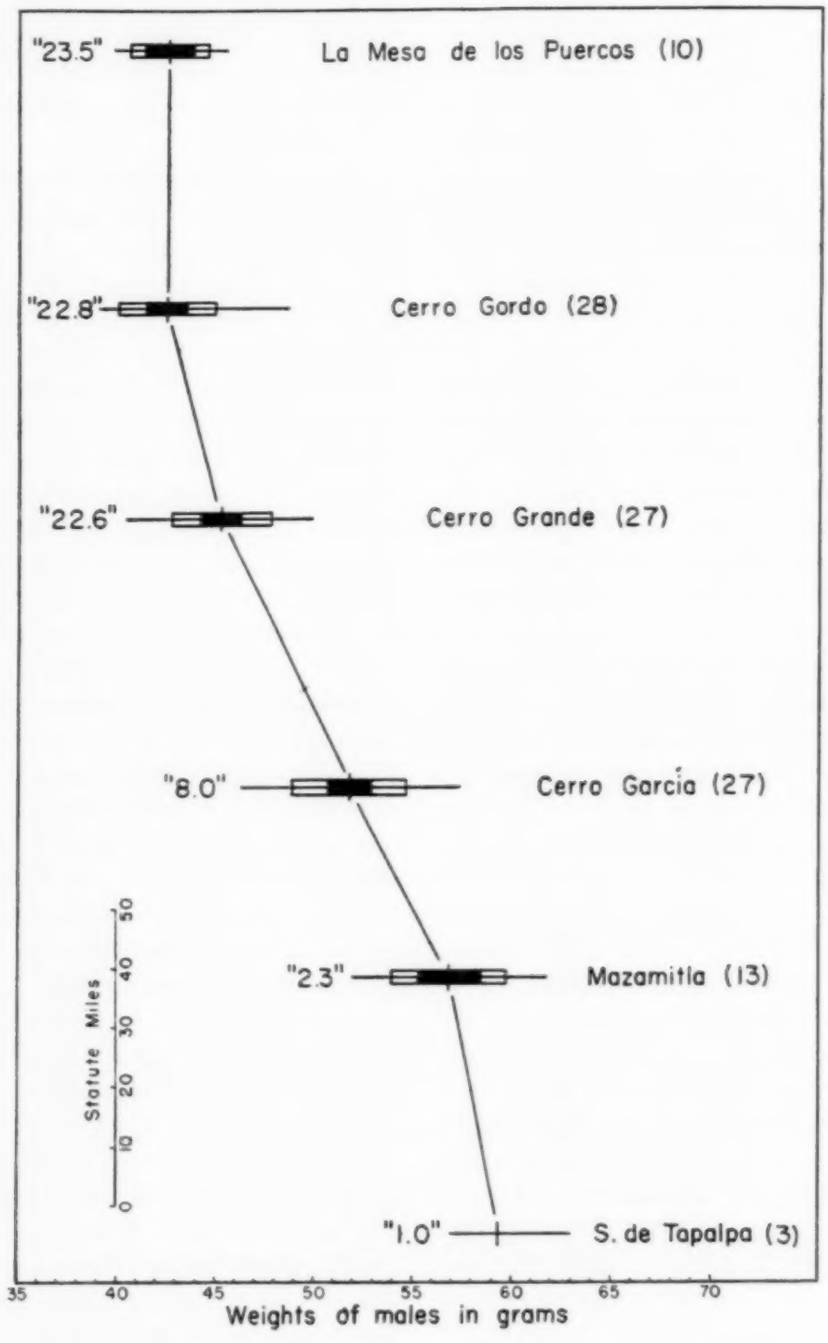

FIG. 9. Statistical analysis of the weights of hybrid male red-eyed towhees from certain localities in western Mexico. Numbers in quotes are average hybrid indices based on color; numbers in parentheses indicate number of specimens in sample. Horizontal lines represent range; rectangles mark standard deviation with solid black indicating twice the standard error of the mean. The means are indicated by a vertical line. The distance between samples is proportional to actual distance in air line miles between populations. The angles of the lines connecting the means are therefore an indication of the amount of the shift in weight between populations.

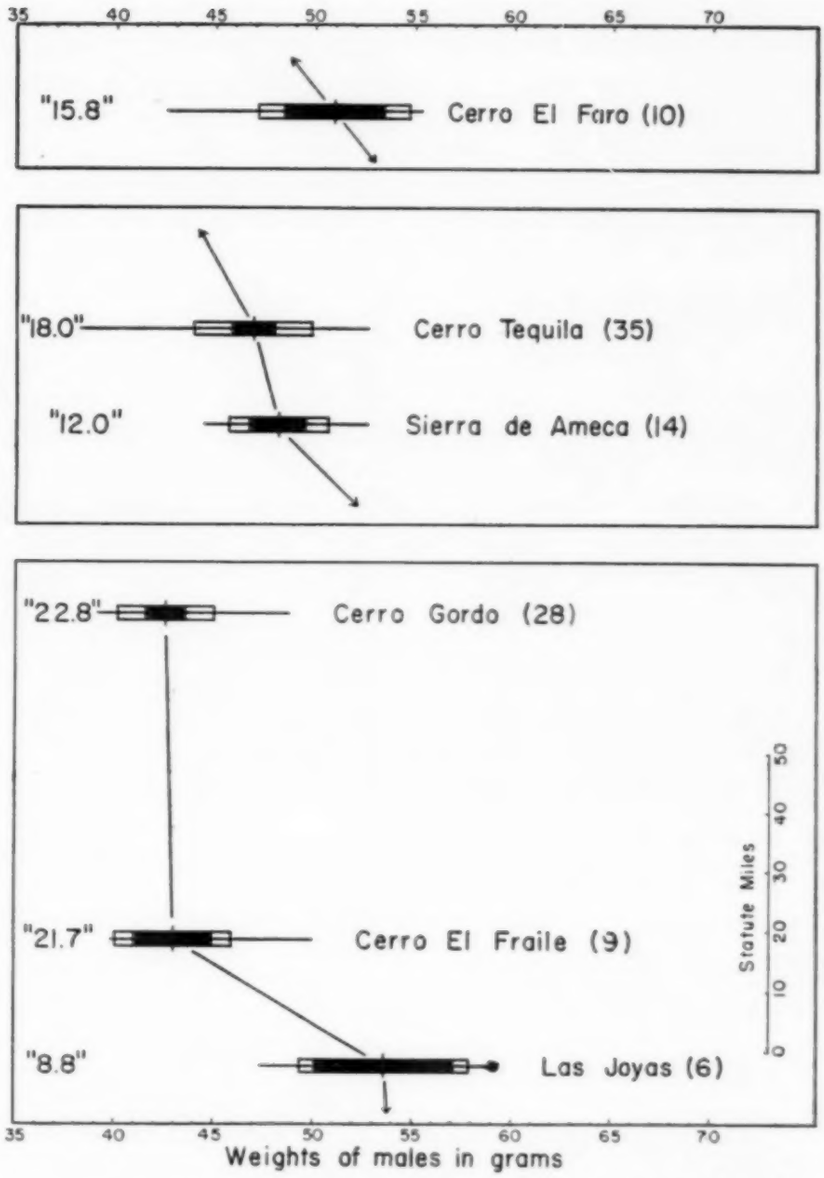

FIG. 10. Statistical analysis of the weights of hybrid male red-eyed towhees from certain localities in western Mexico. See figure 9 for explanation. The arrows indicate the direction toward the mean of the next population sample.

3. Sympatric at Puerto Morelos, Vera Cruz with evidence of a low incidence of introgression.
4. Sympatric in a narrow belt on the west slope of Mount Orizaba above the town of Chalchicomula. Evidence of hybridization found in 15 of the 115 specimens from this locality.
5. A partially isolated hybrid population occupying an area of favorable habitat in the vicinity of Tezuitlán, Puebla which has an average hybrid index of "13.0".
6. Populations of *P. erythrophthalmus* in Hidalgo (Real del Monte, etc.) and Puebla (Honey, Beristain) which show the effects of introgression and have an average hybrid index of "22.4" (Sibley, 1950: 130–134 and figure 18).

THE ANALYSIS OF WEIGHT

The best single index to the size of an animal is its weight. The relatively large difference in weight between pure populations of *Pipilo erythrophthalmus* and *Pipilo ocai* provides a useful quantitative

index for the analysis of hybrid populations and the demonstration of introgressive gradients.

A series of 14 males of *Pipilo erythrophthalmus orientalis* from the Sierra Madre Oriental (San Luis Potosí; Nuevo Leon) range in weight from 35 to 50 grams with an average of 42 grams. Nineteen females from this population range from 35 to 43 grams, averaging 39 grams.

Twenty-three males of *Pipilo ocai brunnescens* from Cerro San Felipe, Oaxaca range in weight from 56–69 grams, averaging 61 grams. Thirteen females range from 50 to 67 with an average of 59 grams.

These populations are phenotypically "pure" and analysis of the above data shows nearly a 30% difference in average body weight between them. The difference between the sexes within each species is of the order of magnitude commonly found in passerine birds. Since the weights of both sexes in all populations follow the same trend it will be necessary to utilize only the males in the analysis.

Figure 9 graphically indicates the trend in weight along the hybrid gradient between La Mesa de los Puercos and the population of *P. o. alticola* in the Sierra de Tapalpa. The correlation with the gradient based on plumage color and expressed as a hybrid index is indicated by the numbers in quotation marks on figure 9 and by comparison with figure 6.

The pictorialized scatter diagram (figure 11) based on some of the populations of this cline also clearly demonstrates the correlation between weight and color characters.

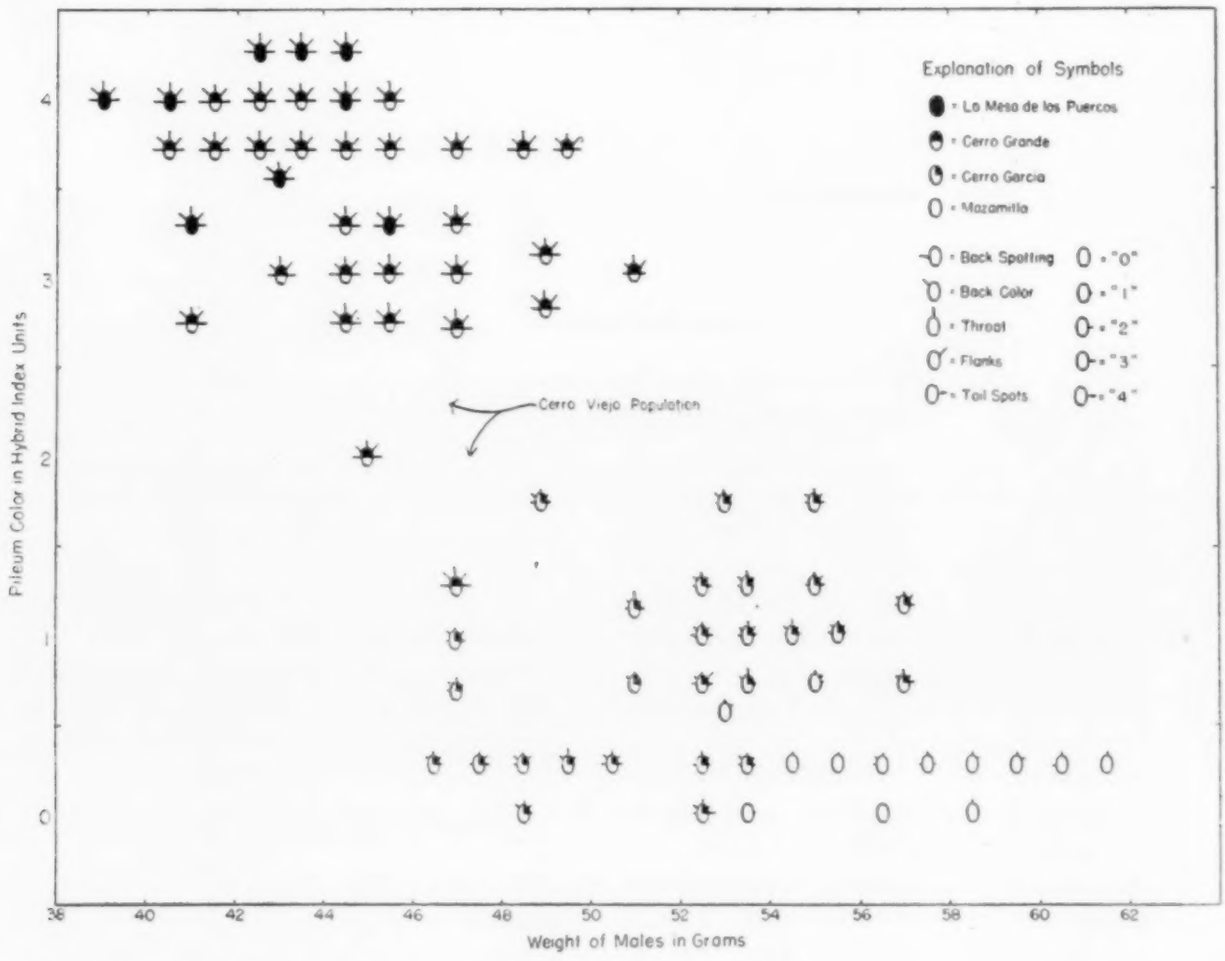

FIG. 11. Pictorialized scatter diagram illustrating the correlation between weight and plumage color for some towhee populations in southwestern Mexico.

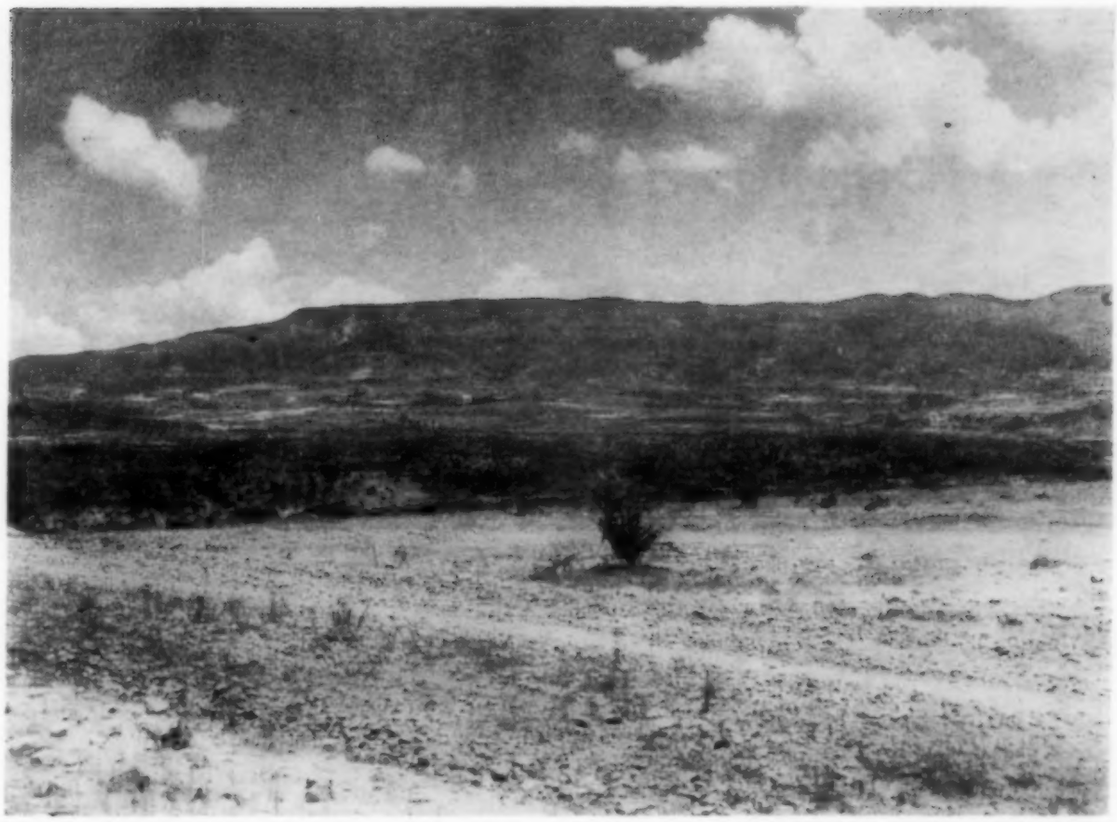

FIG. 12. Looking west from near Yahualica, Jalisco toward La Mesa de los Puercos. Desert vegetation in foreground. Pine-oak woodland on the distant ridges.

FIG. 13. Looking southwest toward Cerro Gordo. Cactus and cornfields in the foreground. Oak woodland on the hill.

FIG. 14. Looking northeast across Lake Chapala from 8,500 feet altitude on Cerro Garciá Oak-madrone woodland in foreground.

FIG. 15. Pine-oak woodland near Mazamitla, Jalisco.

FIG. 16. Looking southeast from Cerro El Fraile toward Las Joyas.

FIG. 17. The vegetation at Las Joyas, Michoacan.

FIG. 18. Looking southwest from approximately 4,500 feet altitude toward Cerro Tequila. Semi-arid scrub vegetation in the foreground. Pine-oak woodland on the mountain.

FIG. 19. Looking south toward the Sierra de Ameca. Cactus, mesquite and cornfields in the foreground. Pine-oak woodland on the mountain.

Weights for the specimens from Cerro Viejo were, unfortunately, not obtained. This makes it impossible to assess the barrier effect of the Río Santiago in terms of weight as has been done in terms of the shift in the hybrid index for color (see p. 267). However, reference to figure 7 and figure 10 will show the striking correlation between the shifts in both weight and color between the populations of Cerro El Fraile and Las Joyas. See p. 272 for a discussion of the explanation for this abrupt change. The parallel trends in weight and color are again demonstrated by the Cerro Tequila and Sierra de Ameca populations (figures 8 and 10).

In summary; the shifts in both weight and color in the hybrid populations forming the segments of introgressive gradients show a high degree of correlation. Since weight is a definite quantitative character this correlation may be taken as a corroboration of the validity of the relatively crude hybrid index used for color analysis.

Discussion

The origin of the patterns of variation which have been described are due, without doubt, to hybridization and the establishment of character gradients through introgression. It seems unnecessary to belabor this point further but it is of interest to attempt to reconstruct the series of events which have led to the complex situation which exists today.

That *Pipilo ocai* and *Pipilo erythrophthalmus* are closely related is apparent. This indicates that in the past they diverged from a common stock and, during isolation, achieved a high degree of morphological difference in color and size. Judging from their present ecological preferences the two species were, under primeval conditions, in large degree ecologically isolated from one another. The breakdown in the ecological separation probably came about with the clearing of the natural vegetation by man as agriculture and lumbering became important. This has occurred within the past 300 years. That the hybrid populations of towhees are not extremely old is suggested by their great variability. If the hybrid populations had become established long before man's modification of the environment it seems probable that selection would, by now, have established a chain of intermediate populations whose variability would be little, if any greater than that of the parental types. Since the only major environmental changes of the recent past are due to the activities of humans it is logical to assume a causal relationship.

Following extensive clearing of the virgin forests the brushy second growth provided optimum environmental conditions for both species and brought them into contact along more extensive fronts than ever before. Because the principal barriers to interbreeding seem to be ethological, and not physiological, it was only a matter of time before a mixed pair was formed. From both natural and aviary observations it is known that when the choice of a mate is restricted many birds will form a pair bond with a member of another species. If this occurs between members of genetically compatible species, a hybrid results. If this is what happened in the present instance, the question is, where? The most likely point for the initial breakdown, judging from the present pattern, was someplace in the "trans-plateau gradient." The exact location of the first hybrid population may never be established but it may have been near Lake Pátzcuaro, as has been previously postulated (1950: 178). Introgression, resulting from the initial breakdown, has spread the genes of both species through contiguous populations. The results are seen today as the hybrid gradients and hybrid mountaintop populations which have been described. The effects of introgression, to date stopped by the Río Santo Domingo barrier, have not yet promoted hybridization in Oaxaca. There seems to be no reason to assume that the Oaxaca populations *can* not interbreed but only that they *have* not. It should be

of interest to sample the Cerro San Felipe populations at intervals and perhaps some future investigator will be able to establish the time of the appearance of the first hybrids and the subsequent history of the situation at that location.

Another question of interest is, how far from the present hybrid populations can introgressive effects be detected? The pattern in Mexico has been described but how far north in the populations of *P. erythrophthalmus* can be found the characters of *P. ocai?*

The distinctive rufous pileum of *P. ocai* is expressed strongly in all hybrid populations in Mexico. The same distinctive color shows up in the otherwise black pileum of *Pipilo erythrophthalmus* as far north as Canada and throughout the eastern United States! If this were due solely to introgression from the hybrid areas in Mexico we should expect to find a higher percentage of *erythrophthalmus* with rufous in the crown in the southwestern United States than in the northwestern states. Of 145 specimens from California, Nevada, Utah and Colorado, 19 or 13%, have some degree of rufous in the crown while of 112 birds from Oregon, Washington, Idaho, Montana, the Dakotas and southern Canada, 12 or 10% show rufous tips or streaks on the crown feathers. These differences are not significant and in the Canadian birds alone six out of 36 or nearly 17% have some rufous in the crown! Thus, instead of answering the question these data merely ask another. Is the rufous in the crowns of northern populations of *erythrophthalmus* due to introgression from *ocai* or to the occasional expression of "ancestral" genes—or to both? At this time the data are too few to provide a basis for further speculation.

The final question is also without an unqualified answer. We still must ask, what is the significance of introgressive hybridization in evolution? It seems probably that experimental studies on plants or rapidly reproducing animals will shed the most light on this problem but some indications are to be derived from the present study. Judging from the high populations of hybrid towhees at many localities it seems that mixed parentage confers no great disadvantage on these birds. The hybrid populations are mostly located in areas in which the natural environment has been altered by man. In plants it has been repeatedly pointed out (Anderson, 1948; 1949: 9) that disturbed habitats provide new environmental niches to which hybrid individuals may be pre-adapted. Birds are seldom as restricted in their tolerance limits as are plants but it is likely that the same principle applies to both. The explanation for the unusually high populations of towhees on Cerro Viejo and Cerro Gordo may well be that the expansion of the genetic latitude of these populations due to hybridization has permitted the occupancy of a wider range of habitats. Nevertheless, the presence of dense, brushy groundcover is a requirement which even the most highly variable populations have not transgressed. In the Sierra de Ameca, which supports a towhee population with an average hybrid index of "12", there is a paucity of brushy cover and a correspondingly low population of towhees.

The demonstration by Smith (1954, in press) that it is possible to obtain hybrid offspring which transgress the range of variation of the parents in at least one character is of prime importance in the assessment of the role of introgressive hybridization as a factor in evolution. Such transgressions in nature would be indistinguishable both in appearance and in effect from mutations. Mutation rates for relatively few genes have been determined and these are mostly lethals. Dobzhansky (1951: 58) reviews the subject and indicates that the evidence concerning the rates of minor mutations responsible for continuous variability is incomplete and contradictory. Until such data are available the over-all relative importance of mutation and introgression must remain in the realm of debate. It is apparent however, that in those situations where hybridization is producing great numbers

of fertile backcrossing individuals that the expanded variability resulting is almost entirely due to introgression. It also seems true that in no known instance has mutation alone produced a range of variation in a short time comparable to the remarkable variability in introgressing populations.

Mutation is important as a source of variability in all organisms. Introgression becomes important only when hybridization is possible. The importance of introgression is thus proportional to the frequency with which fertile backcrossing hybrids are formed. The discovery, analysis and description of hybrid populations is therefore an important phase of evolutionary research.

Summary

The Spotted Towhee (*Pipilo erythrophthalmus*) and the Collared Towhee (*Pipilo ocai*) are morphologically distinct and show marked, but overlapping, ecological differences. In at least one locality, in the Mexican state of Oaxaca, they are sympatric with no evidence of interbreeding as yet obtained. At other points of contact on the Mexican plateau varying degrees of hybridization are found. Several hybrid gradients, apparently resulting from introgressive hybridization, have been discovered. Introgressive color clines are found to be concordant with clines in size (weight).

The patterns of variation suggest that the two species, originally ecologically separated, were brought into contact when forests were cleared for human purposes. An original breakdown of the incomplete physiological barriers promoted continued hybridization through introgression.

The importance of hybridization as a factor in evolution is probably greater in plants than in animals but the increasing number of animal groups in which fertile backcrossing hybrids are being found indicates that the phenomenon is not as rare as once believed.

Acknowledgments

The 1950 field work was made possible by a grant from the Penrose Fund of the American Philosophical Society. To my students and companions, Andrew C. Browne, Eugene A. Olson and Raymond E. Williams, who assisted in the field work, I express my sincere appreciation. Mr. E. R. Blake of the Chicago Natural History Museum kindly loaned the specimens from Rancho San Miguel, Vera Cruz and Mr. Melvin Traylor provided the information on the locality. Dr. Harold H. Smith kindly provided the quotation from his paper which is in press. Mr. Douglas M. Payne assisted with the preparation of the illustrations. My wife has helped with the measurements, the statistical computations and the illustrations.

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NOTES AND COMMENTS

POPULATION SIZE AND EFFECTIVENESS OF SELECTION

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The very interesting paper by Kurtén (1954, Evolution, **8**: 75) contains a statement which, while not leading to any incorrect inferences in the context of the article, appears to be based on a not uncommon misapprehension regarding the relation between selection and population size. On page 78 Kurtén states: "From the studies of population geneticists we know that the effectiveness of selection is dependent on the size of the population." It is probably an undeniable fact that population size is an exceedingly important factor in evolution. Its effects have been explored by a number of investigators, in particular by Sewall Wright, but we know of no experimental studies in which the size of the population has been related to the *effectiveness* of selection.

It is true that with very small populations, say of the order of a hundred or less, the effectiveness of selection is attenuated because the population distribution is discrete and not normal. In such cases selection of a given percentage of individuals as parents of the next generation does not produce as great a selection differential as would similar selection in a very large normally distributed population with the same variance. Kurtén, however, is obviously not dealing with such small populations.

Another way in which population size may have an *indirect* effect on the results of selection is through its influence on genetic variability within the population. In a relatively small population chance or drift may compete with selection in determining just what genes are maintained in the population and what their frequencies would be. In addition, the expected value of the population variance will be decreased in relatively small populations because of the same factor. This effect, however, again has no direct application to the case discussed by Kurtén, inasmuch as the smaller populations he discusses always consist of surviving individuals from the larger ones. In such a case there is no opportunity for inbreeding over a large number of generations to reduce the variance of one population with respect to that of the other, although differences in population variances may arise as a result of selective forces.

Usually population geneticists (see e.g. the chapters on the subject in Lush's "Animal Breeding Plans") consider that the three determinants of selection progress are selection intensity, gene frequency, and selection accuracy (i.e. effective degree of heritability). These factors do not depend directly on population size. Nevertheless, Kurtén's assumption that selection in a smaller population would not be as effective as in a larger one may have, in spite of the above consideration, led him to valid conclusions. Thus it is possible that because the individuals of the smaller, or remnant, population are older, selection in them, while promoting an amount of change per generation equivalent to that in younger animals, might be expected to produce smaller gains per year or other time interval. This consideration was first brought up by Dickerson and Hazel (1944, J. Agric. Res., **69**: 459) in relation to livestock breeding programs.

Other factors might also enter the picture. Thus selection among the younger animals might reduce the variance remaining in the cohort at an advanced age (cf. observations on stabilizing selection cited by Kurtén); the proportion of the total population formed by the older animals is smaller; the fecundity may decrease with age, and so forth.

We have deemed it worthwhile to call attention to this point not in the spirit of criticism of Kurtén's conclusions, but only because of the possibility of confusion between the direct consequences of variation in population size on selection effectiveness on the one hand, and the indirect ones operating through different factors of evolutionary significance (mating system, drift, age composition of population) on the other.

COMMENT ON NOTE BY DEMPSTER AND LERNER ON POPULATION SIZE AND EFFECTIVENESS OF SELECTION

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Dempster and Lerner appropriately draw attention to an obscurity in my text. The particular case under consideration is that of a population with diagonal-type survivorship and opposing directions of selection in young and old age groups.

That the direction favoured in the young will gain primarily because these age groups constitute the majority of the breeding population appears to me so obvious as not to need elaboration; that this factor was mentioned in passing only may, however, give rise to the impression that I considered it of secondary importance.

As a *reinforcing* factor I contemplated the effect of chance; it was felt that survival in the dwindling, old age groups might be dominated by chance incidents rather than selection, and thus still more reduce the effect of counterselection pressure. This effect may be negligible, or it may not. Very strong selection pressure on the young age groups may place the old at such a disadvantage that the potential longevity is reduced and the survivorship of the cohort becomes convex, in which case the factor in question would apparently lose all importance. The actual significance of this and other interesting possibilities can only be established by synthetic work.

For the rest I find myself in agreement with Dempster and Lerner. The discussion, incidentally, shows that some ground has yet to be covered before ecologists and geneticists speak the same language. Aside from the present matter, for instance the term "population dynamics" is now commonly used in two quite different senses, as was the word "mutation" some time ago.